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GLOBAL SURVEY OF RESEARCH AND CAPABILITIES IN GENETICALLY ENGINEERED ORGANISMS THAT COULD BE USED IN BIOLOGICAL WARFARE OR BIOTERRORISM

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This report summarizes the scientific principles underlying genetic modification of microorganisms. It assesses the global availability and cost of technology (know-how, keystone equipment, and synthesized biological materials) needed to acquire and produce potentially lethal biological agents in quantity. The analysis includes technology to engineer novel organisms previously unknown in nature, to synthesize organisms *de novo*, or to modify naturally pre-existing organisms. The document considers the growing proliferation of legitimate activities, and how such activities may enable nations or non-traditional actors to produce genetically engineered infectious agents for uses that could impact national United States or international security.

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SUMMARY

This work summarizes the scientific principles underlying genetic modification of microorganisms. These principles and their enabling methodology are within the grasp of advanced college or graduate students.

We discuss that genetically engineered organisms are characterized as follows:

- assembled from DNA pieces purchased from DNA foundries without ever needing access to virulent agents
- obtained by modifying natural non-virulent organisms
- produced in quantities sufficient to generate potentially high offensive consequences after a relatively small investment (near 100,000 U.S. dollars)
- produced with the research capabilities (as demonstrated by related publications) within any one of a large number of countries where it would be difficult to discriminate offensive from legitimate activities.

Co-analysis of related research and economic indicators suggests that the interest of several countries in genetic engineering has outpaced the national growth of their respective economies.

As a result of investment focused in biodefense, increased research output, vastly expanded genomic databases, and overall national attention, our analysis suggests that the global risk for development of weapons and eventually mounting an attack with genetically engineered organisms of catastrophic consequences is higher today (2008) than it was in September 2001.

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PREFACE

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GLOBAL SURVEY OF RESEARCH AND CAPABILITIES IN GENETICALLY ENGINEERED ORGANISMS THAT COULD BE USED IN BIOLOGICAL WARFARE OR BIOTERRORISM

1. SCOPE

This report summarizes the extent to which technologies for producing genetically engineered infectious organisms are available worldwide. The analysis includes technology to engineer novel organisms previously unknown in nature, to synthesize organisms *de novo*, or to modify naturally pre-existing organisms. The document considers the growing proliferation of legitimate activities, and how they may enable nations or non-traditional actors to produce genetically engineered infectious agents for uses that could impact national United States or international security.

This study briefly describes the most common approaches to engineer microorganisms. Then, it focuses on capabilities, investment, and related research activities from a global perspective. Emphasis is made on demonstrating that access to the actual microbial agents may not be necessary because gene synthesis has advanced to the point where complete sequences of infectious agents (particularly viruses) can be accomplished. Although the threat of genetically engineered organisms (GEOs) is reviewed, threat assessment is outside the scope of this analysis because we neither address specific threat organisms nor speculate on intent. In this study, we do not describe novel potential scenarios for an attack, because involvement of GEOs would worsen any imaginable scenario. In addition, we believe that scenario development often brings attention to issues that weaken national security. The main objective of this work was to provide decision makers with insight into global capabilities and trends that might be exploited to produce novel biological threat agents. Nothing can or should be inferred regarding the willingness or intent of any country or organization discussed herein to undertake production or use of biological threat agents. However, our analysis may point to activities that, if monitored and evaluated, might reveal potential threats.

2. INTRODUCTION

Molecules known as nucleic acids encode the genetic instructions used in the development and functioning of all known living organisms. Deoxyribonucleic acid (DNA) is the genetic material in bacteria, plants, animals, and man. Ribonucleic acid (RNA) is the genetic material of many viruses. Specifically, DNA and RNA serve as long-term "storage" for the information needed to carry out biological processes and construct other biomolecules, such as messenger RNA and proteins. The reader already familiar with microbial genetics or molecular biology could skip this section and still understand the following sections. The reader desiring an in-depth presentation of the scientific basis underlying this document is referred to the excellent texts on microbiology [Talaro and Talaro, 1996; Murray et al., 1999]; virology [Knipes and Howley, 2001; Flint et al., 2004]; and genetics [Rothwell, 1993; Snyder and Champness, 1997] listed in the Literature Cited.

The DNA molecule consists of sugar, phosphate, and four nitrogen bases: adenine (A), thymine (T), guanine (G), and cytosine (C). The nitrogen bases are paired together in specific ways. These connect to the sugar and phosphate backbone, forming a ladder type structure famously known as the "double helix". The RNA resembles DNA with relatively small chemical modifications (one extra OH) in the molecular backbone. The order of the bases, known as their sequence, specifies and directs the production of amino acids, the structural elements of proteins. Proteins are biochemical units that serve a wide range of biological functions and processes, including those that produce disease symptoms. Bacteria and viruses display natural mechanisms for gene transfer and DNA (or RNA) replication. The current capabilities to genetically modify organisms are the result of a remarkable confluence of recent advances in different fields of science. Each of these advances, in its own right, was revolutionary. They have occurred, for the greatest part, since the 1950s, and the rate of technological development escalated dramatically in the past decade. Specific keystone events include the following:

- Discovery of the DNA molecular structure and the role of the double helix structure in genetic inheritance (Watson and Crick) in 1953

- Success of Celera Genomics, Inc. in using the so-called "shot-gun" approach to assemble the human genome in 2000

- A 12 order of magnitude increase in the raw computational throughput of state-of-the-art digital computing, multiplied in recent years by the explosive growth of interconnection capabilities, including the Internet.

- A rapidly expanding suite of instruments and tools for observing, measuring, and manipulating matter and reactions at nanoscale and atomic levels. These, in turn are providing unprecedented capabilities for high resolution assay and analysis of DNA, proteins, antibodies, and other biomaterials.

The result of these and related advances bring an unprecedented capability for understanding and visualizing biomolecular processes and for manipulating matter at molecular and atomic levels. Science has exploited these technical advances to alter natural organisms by using recombinant DNA (rDNA) technology. Recombinant DNA is a technology (summarized in Figure 1) that allows portions of genetic material from another organism (generally small segments of DNA such as bacterial plasmids) to be introduced into a living organism (the host).

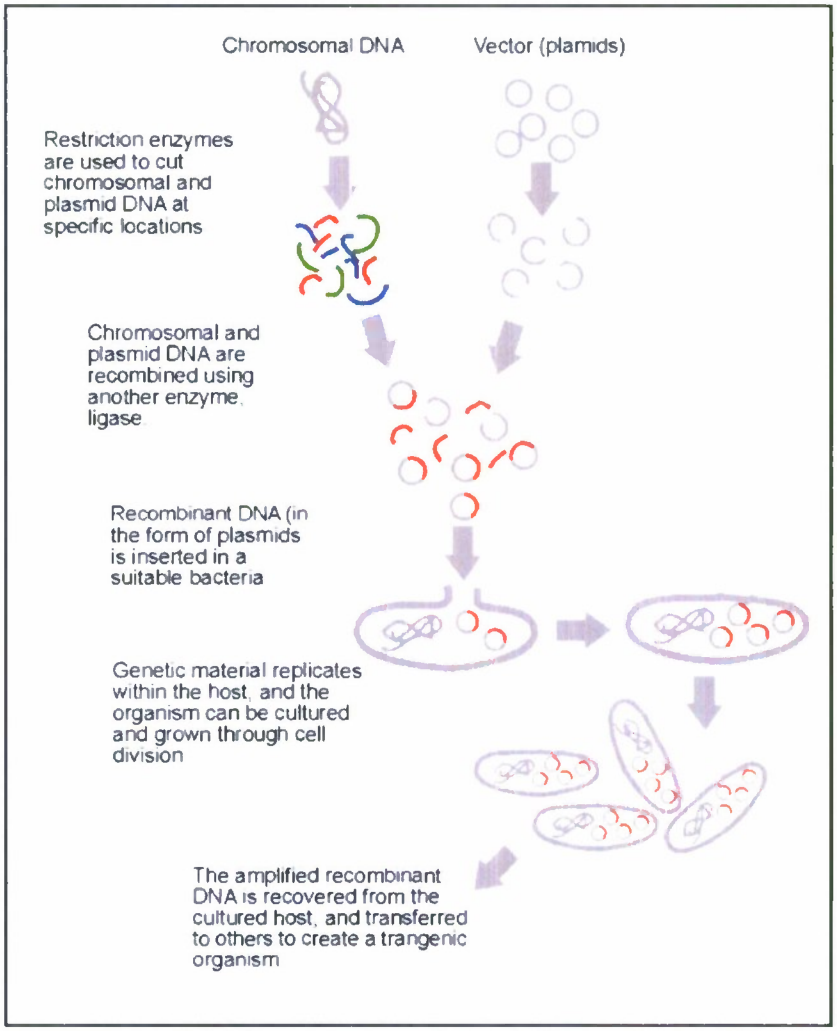


Figure 1. Overview of rDNA

The inserted gene/s directs replication of specific DNA sequences with a concomitant expression of desirable characteristics in the host organism. Thus, the genetic material of the host organism (generally called a vector) is recombined with genetic material from another organism. Viruses can also be used (as vectors) to transfer genes useful to mankind, which makes virus engineering a genuine peaceful activity.

Standard or "classical" genetic engineering of novel recombinant genomes can be envisioned as one of "cutting" and "pasting" specific genes into organisms where the genes were not naturally occurring. Thus, it is possible to artificially mutate organisms, introducing either new or modified genes into an organism to manipulate its characteristics.

Several steps in standard rDNA methodology can be either simplified or eliminated altogether by copying DNA through a process of enzymatic amplification such as the **polymerase chain reaction** (PCR), which does not require living organisms. (An overview of the process can be found in Wikipedia (http://en.wikipedia.org/wiki/PCR). The current state of the art in PCR allows for amplification of either DNA or RNA fragments as long as 40,000 bases (Reisinger et al., 2006), a size which is substantially longer than the genome of many infectious

viruses (e.g., the genome of Ebola is approximately 19,000 bases long [Volchkov et al., 2001]. Then, the PCR can be used to generate complete viral genomes from a template that can be modified to result in virus progeny with different properties.

In addition to standard rDNA technology, there are other approaches to produce engineered organisms. Modified bacterial chromosomes amplified by PCR can efficiently recombine (a method known as "**recombineering**" [Thomason et al., 2007]), allowing DNA sequences to be either inserted or deleted as desired. **DNA shuffling** [Stemmer, 1994] or **molecular breeding** [Soong et al., 2000] mimic natural processes of recombination that lead to new mutations or phenotypes; but, these artificial processes produce genetic changes at a vastly accelerated pace. **Reverse genetics** can produce infectious viruses from full-length cloned DNA by cellular polymerase synthesis (Chang-Wong Lee et al., 2008]. Synthesis ***de novo*** allows chemically synthesizing of new viruses (or resuscitating old ones, and eventually also bacteria) by simply keying either a natural or modified genome sequence into an automatic synthesizer.

Once the rDNA, new gene, or whole novel genome has been generated and introduced into either one or a few host organisms by any available approach, the novel DNA can be "amplified" by several methods. The most common are either through cultivation and growth of the host organism containing the recombinant insert, or through a process of enzymatic amplification such as PCR (as indicated above). In addressing the issue of biosynthesis of select agents, the National Science Advisory Board for Biosecurity (NSABB, 2006) indicates that "Synthetic Genomics": "...generally refers to the design and production of viral genomic DNA or RNA for the purpose of expressing the encoded viral product. Because of technical challenges, expression of bacteria and other larger life forms from synthetic genomes is not currently feasible; however, efforts are underway to achieve these goals."

In our analysis, the term "genetically modified organism (GMO)" (also referred to as transgenic organism) denotes an organism whose genes have been altered by deliberate (human versus naturally) insertion, modification, or deletion of genetic material. The GMOs typically involve the splicing of naturally occurring genes selected for specific properties. The GMOs here are considered to be products of human manipulation. This distinction is needed to differentiate GMOs from all other organisms that evolve by naturally occurring genetic modifications (mutations). Natural mutations are seen in the growing number of antibiotic-resistant bacteria as well as in the treatment-resistant strains of Human Immunodeficiency virus, which causes AIDS. "Genetically engineered organisms" (GEOs) include (i) GMOs; as well as (ii) natural or chimerical organisms synthesized *de novo*, even having a naturally existing genome (as in influenza virus); (iii) produced by combining genes from diverse organisms (like chimeras containing viral and bacterial genes); or by (iv) re-assorting into a different strain genes naturally occurring on another (generally virulence genes). Actual gene manipulation involves rDNA techniques; but, the design of potentially dangerous GEOs requires an understanding of genomics. The field of genomics is generally divided into three major sub-areas:

- **Fundamental Genomics** — where research is generally directed towards mapping the basic structure of human and microbial genomes. Understanding the basic structure, organization, and function of human and microbial genomes potentially allows development of genetically modified threat organisms resistant or immune to the natural defenses of the human body. This field witnessed significant breakthroughs in the early 2000s; for example, when Australian researchers discovered (serendipitously) that altering a gene in mousepox virus (a virus relative to smallpox) unexpectedly killed all the experimental mice by destroying the mouse immune defenses [Jackson et al., 2001].

- **Functional Genomics** — Once the structure of a genome is determined (sequenced), a major task remaining is to determine the function of each of the genes. Science is just beginning to develop this knowledge base. The effort is complicated by the following facts:

    - Only part of the total DNA structure appears to have a defined function.
    - Different genes within the genome are turned on and off in different sequences and combinations to perform different functions. The old "one gene, one protein" model is obsolete because genes have multiple roles and do multiple things.

- **Proteomics** — The complex set of proteins encoded by a cell during its lifetime is referred to as the proteome. One of the ultimate objectives of genomics is to understand, in detail, how the different genes encode for the synthesis (also known as expression) and assembly of proteins. In addition to being relevant to the understanding of biological processes, complete understanding of this aspect of genomics would, in theory, permit researchers to develop new processes and biomaterials. For example, controlling the protein expression of antigenic proteins in a microorganism could make such a microorganism resistant to vaccines or undetectable by antibody-based diagnostics. Also, GEOs could be tailored to either express proteins or metabolize products that would be toxic to the host.

The underlying technologies for developing and exploiting genomic information can be divided into broad areas that support the three sub-fields of genomic research described above, including the following:

a. **Gene sequencing** is the basic "pick and shovel" work of determining the physical structure of the genome.

b. **Molecular biology and biochemistry**, including technologies for rapid screening and combinatorial chemistry, are essential to advancing functional genomics, and to understanding the biological and chemical effects of specific proteins and other biochemicals expressed during biological processes.

c. **Protein engineering and bioprocess engineering** extend molecular and genetic knowledge to optimize large scale and affordable production of organism or biological materials.

d. Bioinformatics is a critical developing field comprised of state-of-the-art and entirely new information processing capabilities, which are required to make effective use of the vast volume of data produced by biomedical research. Bioinformatics can identify metabolic pathways from specific gene to end product, molecular structure, and even correlate gene sequences of threat viruses to the disease that they cause.

However, decoding and building the full database of genetic functions is significantly more complicated than sequencing the genes as in (a) gene sequencing above, (b) mapping biochemical functions to particular genes, or (c) producing organisms or their products in large quantities. Managing the large amount of information codified in the genome of microbial and larger organisms has developed the field of (d) bioinformatics. Bioinformatics' ability to manage large amounts of data assists in understanding interactions among genes and their control functions. Thus, bioinformatics is the key to understanding the pathways whereby infectious agents produce their deleterious effects. Hence, once the functions of microbial genes are known (a), their interactions with their (human) host identified (b), and the infectious processes are understood (c), disease outcomes can be ameliorated or enhanced, thus being controlled for good (health) or evil (warfare). Fortunately, most of the relevant research in genomics is driven by health and medicine. However, the field of genomics encompasses a wide range of activities having potentially significant military impact.

3. THREAT OF GENETIC ENGINEERING

Engineering microorganisms, such as bacteria and particularly viruses (whose smaller genome makes them relatively easy to manipulate) are genuine peaceful activities that benefit many aspects of society. A matter of concern in this analysis is that such GEOs may be tailored to avoid detection, circumvent vaccines or antimicrobial therapy, augment infectivity and virulence, accelerate onset of disease, or increase the mortality rate. The concern that GEOs may be exploited by either terrorists or antagonistic states to produce biological threat agents has been recognized for a long time [NSABB, 2006].

Because developing GEOs for offensive purposes would be simplified by starting with already virulent threat agents, The Select Agent Rules (implementing the USA Patriot Act and Public Health Security and Bioterrorism Preparedness and Response Act of 2002) set requirements for the possession, use, and transfer of a number of pathogenic microorganisms and toxins [Federal Register, 2005]. These U.S. regulations (and their counterparts in relatively few other nations) do not apply globally.

Even if the Select Agent Rules or other legal deterrents for possession of threat agents would be globally accepted, the substantial foreseeable efforts that would be required for world-wide enforcement would still be inadequate to prevent access to threat organisms that periodically and naturally emerge or re-emerge at different parts of the world. Examples include the Influenza virus, Ebola virus, or any of the South American hemorrhagic viruses, as well as emerging antibiotic-resistant bacteria (like antibiotic-resistant tuberculosis) or novel bacteria [like the ones responsible for Severe Acute Respiratory Syndrome (SARS) in 2003]. All of these could be amenable to genetic modification. Such potential agents are accessible to largely

anybody willing to travel to endemic areas. Thus, the ability of government bureaucracies to enforce any rules in many parts of the world where deadly biological agents periodically arise is questionable.

The growing body of functional genomic and proteomic information that allows designing practically any infectious agent that can be envisioned is undeniable. In addition to concern for the relatively easy access to threat organisms from natural outbreaks, modern DNA synthesis technologies now also allow for creation and use of purely synthetic genes. This potential access to virulent organisms, produced by *de novo* chemical synthesis instead of from natural sources, has crucial implications for the potential risk of biological agents. While synthesis of the complete genome for bacteria and larger life forms is not yet feasible, the use of gene modification to circumvent vaccines, defeat diagnostics, enhance the virulence, infectivity, or mortality rate of naturally occurring organisms, particularly viruses, is within the current state of the art. In addition, we will discuss below readily available capabilities in microbiology and genetic engineering that allow producing GEOs without need for access of threat micro-organisms, making some of the Select Agents Rules regarding possession of virulent agents outdated, if not obsolete, to contain a modern biological warfare program.

Safety rules and regulations can be useful guidelines that apply to the legitimate developer of genetically modified agents, to whom safety is a primary concern. In the case of bioterrorists or a determined national warfare program, biosafety may not necessarily be an overriding concern as long as any fallout or other locally undesirable effects can be contained. Because trained microbiologists are essential to any offensive biological program, even terrorist organizations will likely want to protect their scientists. However, acceptable safety for a terrorist organization or a desperate nation may be achieved with relatively fewer and simpler measures than those implemented in developed nations without the need of an offensive biological program. Acceptable protection in a low-budget program may be attained by a combination of individual protection, simple equipment (a laminar flow cabinet, or glove boxes, UV disinfection of clean benches, and air filtering), and careful procedures, instead of expensive infrastructure and costly regulatory bureaucracy.

Medical history has shown that the causative agents of even the most devastating epidemics allow their host, in time, to develop natural responses of increasing resistance and immunity. The threat of malicious man-made GEOs is particularly daunting because it raises the possibility of generating transgenic agents that nature would not normally create, and against which the (human) host may not develop natural resistance. The concern that human genes could be engineered for malignant purposes was highlighted by the U.S. Department of Defense (DoD) in the latest update to Section 4: Biomedical Technology in the Militarily Critical Technologies List [Department of Defense, 2007] as follows:

> The decoding of the human genome over the past decade has resulted in the identification of human genes that, when inserted into non-pathogenic viral particles, can markedly increase the susceptibility of persons exposed to such particles to virulent disease, to loss of cognitive function, and to increase in anxiety. The virulent disease is caused by loss of immune competence

> rather than by exposure to a highly infectious agent of the Australian group. The loss of immune competence is similar to that seen in patients with acquired immunodeficiency syndrome (AIDS) or in transplant patients who are immunosuppressed. The reduction in cognitive awareness is a result in perturbations in the expression of enzymes that regulate neurotransmitter levels.

Although there is a potential risk to confer a depressed human response to infectious GMOs (as proposed by this rather dramatic statement that can be related to the preponderance of the medical component within DoD biodefense activities), there are easier, better known, and more direct ways of inflicting harm. In addition, the practical need for human testing to verify increased human susceptibility to a GMO with genes that supposedly reduce human immunocompetence adds a degree of complexity that should make this immunological approach less attractive as a biological weapon than other approaches.

Many of the most virulent and deadly viruses reside in a natural reservoir between outbreaks of human disease. Examples include the Hanta virus (rodent-borne), Avian flu (birds), and Ebola (where the natural reservoir is believed to be the fruit bat; but, that has, as of this date, not been definitively confirmed) [Sanches et al., 2001]. The implication of this is that, although direct human-to-human infection poses the greatest threat and is a likely objective of genetic engineering, the possibility of modification of a naturally occurring virus to enhance its transmission from a natural reservoir to the human population cannot be discounted.

Emphasis on the risk of GEOs from a medical perspective has focused generally on decreasing human susceptibility (various theoretical approaches to immune manipulation), altering the virulence of microorganisms, increasing microbial resistance to therapy (e.g., antibiotic resistance), or making them able to circumvent vaccines. However, it should be noted that the ability to introduce artificial genes into natural pathogen or chimerical organisms does not directly translate into a usable threat. The GEOs for offensive purposes should be viable, infectious, and hardy enough to withstand environmental conditions and to remain viable for the relatively long period between dissemination and (human) infection. These microbial environmental requirements are not easy to fulfill by GEOs to be produced with the current knowledge, which has developed more extensively in molecular biochemistry than in environmental microbiology.

Although attracting generally lesser attention from the medical establishment, genetic engineering could also be applied to alter non-medical characteristics that may enhance organisms as fieldable weapons. For example, GEOs could be designed to be undetectable by antibody- or nucleic acid-based detectors, temperature tolerant, resistant to decontamination [Sagripanti et al., 2007], less sensitivity to UV radiation [Lytle and Sagripanti, 2005; Coohill and Sagripanti, 2008], more efficiently aerosolizable, or infective by a more efficient route. Compared to altering human resistance to disease, it appears relatively simpler to engineer organisms with any of a series of physico-chemical (non-medical) properties that would make the germ last longer, reach farther, or be stealthier.

It is generally assumed that the level of sophistication required for genetic engineering is within reach only of technologically sophisticated states seeking to develop a biological warfare capability. We will present evidence below suggesting that widespread current scientific knowledge brings the capabilities of the genetic era within reach of the majority of nations (even those at early stages of development), of terrorist groups, and of other non-state actors.

4. KNOWLEDGE IS GLOBALLY AVAILABLE

The knowledge that would be needed to design and produce GEOs for offensive purposes is considerable. For example, it is generally accepted that it is rather difficult to identify, select, and engineer different antigenic determinants to pass undetected antibody-based diagnostics, circumvent vaccines, or express toxins for which little or no natural immunity might exist. However, public health and economic gain have driven global dissemination of the knowledge required for genetic modification for pharmaceutical or agricultural purposes.

The information is freely accessible. The genetic information on a vast number of microorganisms as well as animals, plants, and humans is well structured, largely standardized, and accessible at www.ncbi.nih.gov/Genbank/. The International Nucleotide Sequence Database repository of DNA sequences managed by the National Center for Biological Information (NCBI) exceeded 100 gigabases in 2005 [at the Genbank web site indicated above] (A gigabase corresponds to 1,000,000,000 bases). For comparison, the whole genome of Ebola virus is approximately only 19,000 bases long [Volchkov et al., 2001]. More than 400 large genomic sequencing projects were listed by the NCBI [web site visited in January 2007], with the majority (269) being sequencing projects for bacteria. In addition to the United States, the latest update to the Biomedical Section of the Militarily Critical Technologies List [DoD, 2007] also identifies the following countries as having large scale datasets related to genetic engineering: Australia, Germany, Italy, The Netherlands, Sweden, and the United Kingdom. In addition to GenBank, the DNA Data Bank of Japan (DDBJ) [www.ddbj.ac.jp], the European Molecular Laboratory database [www.embl.org/], and the International Nucleotide Sequence Database (INSD) Collaboration [http://insdc.org, see below] are also substantial repositories of genetic information freely available on line. Thus, basic gene mapping and functional genomic data is widely available from on-line databases. As a result of the free exchange of technical information, the quantity of gene mapping information has grown exponentially in the past decade, and is ubiquitously available.

The Web also provides access to bioinformatics applications and software tools for analyzing genomic information; often, much of the data and software can be downloaded free. Free and open access to these bioinformatics tools allows, in principle, to obtain the information to design (but not actually synthesize or produce) GEOs with desirable properties. There is also a growing body of knowledge and data on proteomics, thus linking nucleic acid sequence information with protein biological function. These databases and tools characterize the function of specific genes with ever-increasing detail and fidelity.

The European Bioinformatics Institute [www.ebi.ac.uk] (EBI), part of the European Molecular Biology Laboratory (www.embl.org/), provides on line access to a comprehensive range of tools for the field of bioinformatics (over 135 are currently listed). A few examples include:

- Similarity and Homology - the BLAST or Fasta programs can be used to look for sequence similarity and infer homology.

- Protein Functional Analysis - InterProScan can be used to search for motifs in a protein sequence of interest.

- Proteomic Services NEW - UniProt DAS server allows researchers to show their research results in the context of UniProtKB/Swiss-Prot annotation.

- Sequence Analysis - ClustalW a sequence alignment tool.

- Structural Analysis - MSDfold or DALI can be used to query any protein structure and compare it to those in the Protein Data Bank (PDB).

- Web Services - provide programmatic access to the various databases and retrieval/analysis services EBI provides.

- Tools Miscellaneous - Expression Profiler a set of tools for clustering, analysis, and visualization of gene expression and other genomic data.

Answers to most technical questions that may rise can be found on the World Wide Web at one of the help sites from the many universities that carry related activities within newly formed departments of Bioinformatics or specific Informatics Resources. In particular, the INSD is the result of a collaboration of three major microbiological data bases:

- The European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database (EMBL-Bank) (part of the European Biotechnology Initiative)

- The NCBI GenBank

- The DDBJ

The INSD Collaboration has a uniform policy of free and unrestricted access to all of the data records their databases contain. Scientists worldwide can access these records to plan experiments or publish any analysis or critique. Appropriate credit is given by citing the original submission, following the practices of scientists using published scientific literature.

Their policy further states that the INSD will not attach statements to records that restrict access to the data, limit the use of the information in these records, or prohibit certain types of publications based on these records. Specifically, no use restrictions or licensing

requirements will be included in any sequence data records, and no restrictions or licensing fees will be placed on the redistribution or use of the database by any party.

This discussion demonstrates that the sequences of threat biological agents are freely available, and the information needed to synthesize or modify them is freely accessible. Anyone may access the computational tools to design GEOs free of charge.

Relevant technical advances have been achieved in many countries. Several such reported achievements of direct relevance to this analysis and freely available in the literature include the following:

- Cloning of a full-length virus genome (Poliovirus) that was infectious to mammalian cells [Racaniello and Baltimore, 1981, United States].

- Generation of a whole infectious genome (of a plasmid 2.7 kilobases long) from a pool of synthetic oligonucleotides [Stemmer et al., 1995, United States]. Later generation of a complete infectious genome (5,400 bases long in a bacteriophage) from synthetic oligonucleotides synthesized according only to the sequence reported in GenBank. The synthesis and assembly of this organism were completed in only 14 days and without a need for accessing any living organism [Smith et al., 2003, United States].

- Cloning of Influenza A (a negative sense RNA virus) and expressing viral genes into mammalian cells [Pleshka et al., 1996, United States and Spain]. Improved reverse genetics to produce Influenza A virus with high efficiency [Neumann et al., 2005, United States and Japan]. Efficiency of cloned Influenza A virus further improved [Ozawa et al., 2007, Japan]. Simpler and faster method (using RT-PCR) to generate influenza virus [Wang et al., 2008, China and Canada].

- Modified mousepox virus [in the same family (Poxviridae) as smallpox] to develop a contraceptive vaccine accidentally becomes 100% deadly by circumventing host immune defenses, even in previously immunized (vaccinated) animals [Jackson et al., 2001, Australia].

- Producing a chimerical virus infectious to cells of a new mammalian host starting from natural viral parents that did not infect that host [Nay-Wei Soong et al., 2000, United States].

- Reverse Genetics of Uukuniemi virus (a Bunyavirus) [Flick and Pettersson, 2001, Sweden]

- Cloning and recovery of infectious Ebola virus (and of a mutant more cytotoxic than the natural wild-type) [Volchkov et al., 2001, Germany and France].

- Chemical synthesis of infectious Poliovirus in the absence of natural template [Cello et al., 2002]. *The boundary between live organisms and chemistry may have vanished definitively.*

- Reverse genetics of Crimean-Congo hemorrhagic fever virus [Flick et al., 2003, Canada and Sweden].

- *In vitro* generation of highly infectious synthetic Japanese Encephalitis virus that remained stable for at least 180 generation cycles [Yun et al., 2003, Korea].

- Complete sequencing and resuscitation of the virus that caused the great influenza pandemics and killed 20-40 million people in 1918 [Taubenberger at al., 2005, United States; Tumpey et al., 2005, United States].

- Reverse genetics of Avian influenza virus [Chang-Wong Lee, and Suarez 2008, United States]. Simplified reverse genetics of Avian influenza virus [Shuai Wang et al., 2008, Canada and China].

- Complete genome sequence of 45 variola strains providing supplemental material with gene organization of smallpox, freely available in the web [Esposito et al., 2006, United States, United Kingdom, Germany].

- Procedure to construct *de novo* error-free DNA molecules from error-prone (commercially available) oligonucleotides [Linshiz et al., 2008, Israel], with the potential to allow masking of the intended synthetic molecule or organism during purchase of oligonucleotides.

- Whole genome amplification (originally intended for forensics) is accomplished without the need of oligonucleotides [Li et al., 2008, United States].

The purpose of the list above is not to be an all-inclusive account of every microbe that was engineered or of each technique that has been developed. Instead, the historical sequence of selected events attempts to illustrate the feasibility of reproducing any infectious agent (whose genome be either DNA or RNA, positive- or negative-sense, mono- or multi-segmented, etc.) and of engineering nearly any agent that can be imagined by approaches that have become progressively simpler and faster. There is also a trend for the research to become independent from the need for accessing microbial agents, specific sequences, or synthetic oligonucleotides that could be amenable of tracing.

Without receiving the attention associated with achievements reported with infectious agents (as listed above), research to develop either simulants or surrogates as alternatives to using infectious organisms during the development of detectors, collectors, or diagnostics products is also of importance in our study. Molecules used as simulants in developing countermeasures for a number of select agents have been constructed by engineering sequences readily available on the internet (see above). A single nonvirulent molecule was engineered (relatively easily) with the signature sequences (disease provided in parenthesis) of

Yersinia pestis (plague), *Francisella tularensis* (tularemia), *Burkholderia mallei* (glanders), *Burkholderia pseudomallei* (malieidosis), *Coxiella burnetii* (Q Fever), *Brucella species (brucellosis)*, *Rickettsia species* (spotted fevers and typhus), entherohemnorrhagic *E.coli O157:H7* (food poisoning), smallpox virus (variola), and both plasmids of *Bacillus anthracis* (anthrax) [Carrera and Sagripanti, 2008]. Another nonvirulent chimerical organism was engineered with the genetic signatures of the viruses: Ebola, Marburg, Lassa, Junin, Machupo, Yellow fever, Eastern Equine Endcephalitis, Venezuelan Equine Encephalitis, Rift Valley, Crimean-Congo, Dengue, and Influenza [Carrera and Sagripanti, 2009]. Although nonvirulent simulants have no offensive potential, their construction demonstrates that chimerical molecules containing genomic portions of many different threat organisms can be fabricated without access to any of the virulent agents.

The evidence presented in this section indicates that (1) knowledge and expertise in genetic engineering is the keystone ingredient to produce GEOs; (2) the application of this knowledge is difficult to evaluate because the boundaries of academic research overlap with potential offensive applications; (3) information on gene mapping and sequencing is openly shared, and therefore can not be traced or controlled; (4) key accomplishments in the field, once almost exclusively within the United States, are being achieved now also in other countries, particularly in Asia.

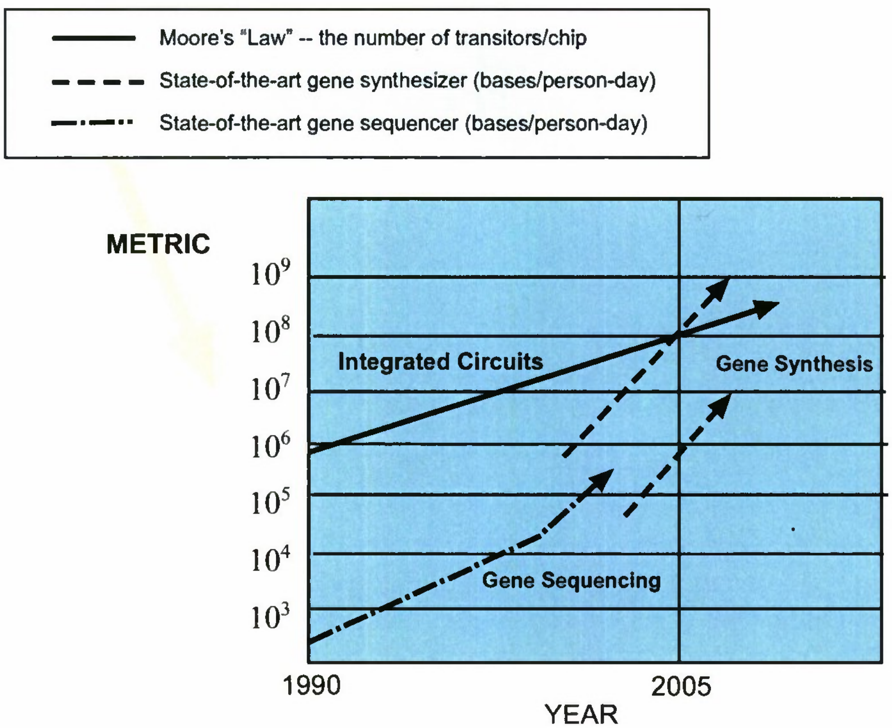
5. GENETIC ENGINEERING CAPABILITIES' RAPID GROWTH AND LOW COST

The key role of microbiology in meeting basic societal needs in health and agriculture provides an intrinsic requirement for some level of expertise in genetic engineering and related activities even in lesser-developed countries. Thus, research and study of all aspects of microbiology can be proper and necessary for advancements in many fields, including public health, food production, environmental remediation, and medical response. All these activities can have dual purposes. Indicators of global capabilities and trends in science and technologies for generating genetically engineered threat organisms will be analyzed later. Primary interests involve gene mapping activities and gene banks related to microorganisms' causative of infectious disease (bacteria, phages, and other viruses), with secondary interests in those repositories incorporating either human tissue or genetic information. This refers to the development and maintenance of databases comprising information on functional genomics or proteomics associated with the human genome and/or infectious diseases.

5.1 Genetic Information Expansion

A key trend in microbial and genetic capabilities is the growing number of research institutes involved in computational biology. Computational biology entails analysis and interpretation of complex genomic and proteomic data, which is key in harnessing genetic information for peaceful as well as offensive purposes. This biology, also known as bioinformatics, exploits the growing availability of high performance computers and powerful search and retrieval algorithms to access, analyze, and manage genomic data. Demand for high performance computing in computational biology is driven by the rapid growth in biological science.

The rate of growth in gene sequencing and synthesis technologies has been compared to an iconic measure of rapid technological advance, e.g., Moore's Law [Carlson R., 2003]. In 1965, Dr. Moore (then at the Intel Corporation) projected that the number of transistors per chip would approximately double every two years [Moore 1965]. The projection has proven to have remarkable staying power. Figure 2 represents an adaptation of the original comparison between DNA and computer chip technology. Although the chart presents two different technologies, the relative rate of growth in genetic sequencing appears to grow at a rate at least similar to, if not faster than, the number of transistors in computer microchips.



The metrics are unrelated, but serve to illustrate that the rate of advance in gene sequencing and synthesis in recent years has outstripped has been historically considered the bench-mark for technological growth, Moore's "Law." Moore projected, in 1965, that the number of transistors on a chip would double approximately every two years.

Figure 2. Comparative Rates of Technological Advances

In addition, biotechnology has several advantages for rapid expansion over the microcircuitry depicted by Moore's law.

(a) While microchips are understood by a relatively small number of experts, the key processes in genetics are within the realm of a more numerous population of students, technologists, and even relatively untrained persons.

(b) The rapid growth of genetic information has resulted in development of the discipline of Bioinformatics devoted specifically to advance data processing, analysis, and understanding of gene functions.

(c) The cost of setting up and operating units of production is very advantageous for biotechnology compared to other enterprises.

5.2 Genetic Engineering Cost

We combined the costs from a number of diverse open sources and compared the typical initiation and operation costs of the different activities shown in Table I. The costs for integrated circuit manufacturing facilities represent the basic cost to construct and startup a new generation integrated circuit line. This data comes from open sources and discussions with individual members of the Department of Commerce Information Systems Technical Advisory Committee (ISTAC) [http://tac.bis.doc.gov/index.html, accessed September 2007]. The ISTAC includes representation from major integrated circuit manufacturing companies. Construction costs of nuclear power plants are widely available on the World Wide Web. Operating costs, which are probably significantly understated, were estimated based on staffing levels for a typical nuclear plant.

Table I. Comparative Costs

Cost in millions of dollars (US)	Integrated Circuit Manufacturing	Nucelar Power Plant	Rural Road, 100 miles paved	State of the art Biotech Research Center	Laboratory Scale Facility
Infrastructure Capital Cost	2000-4000	5000-6000	250	40-100	<0.200
Annual Operating Costs	500	500	60-12.5	10-25	<0.3

Infrastructure Capital Costs include the cost of contructing and equipping the facility or infrastructure element listed. Annual Operating Costs include salaries of staff and expendable supplies, except for the Nuclear Power Plant example, which is based on labor cost only, and is, therefore, arguably substantially understated

Costs for Biotech companies were estimated from news articles in trade magazines, budgeting and planning documents from state and local governments, and academia [Carlson, 2003, Genetic Engineering and Biotechnology News, 2005; Pittsburg Tribune Review, 2007]. Costs ranged from $500 million (U.S. dollars) for construction with $35 million for annual operating costs, for a Biotechnology Center designed for large scale production, to a few $tens of millions for local and academic biological research centers. Based on a broad review of similar resources, the figures shown are representative of what appeared to be typical figures for a state-of-the-art facility capable of supporting large scale development and production of engineered biological agents. The cost of a relatively small scale laboratory facility is discussed below. Finally, costs of constructing 100 miles of rural road are provided for comparison [Florida Department of Transportation, 2007; Travis County-Texas, 2005; Sacramento Area Council of Governments, 2005]. As a result of a comparatively low level of investment required and a relatively high level of demand for licit purposes, the infrastructure and wherewithal for genetic organism manipulation exist on a global scale.

6. LABORATORY INFRASTRUCTURE IS WIDESPREAD

A key indicator of an ability for microbial production and manipulation has historically been the existence (in Government, academia, or industry) of laboratories with high level biological containment (Level 3, and Level 4 laboratories) and/or research hospitals engaged in biomedical research in infectious diseases (Appendix A). The global distribution of known BSL-4 laboratories (as of December 2008) able to safely work with (and genetically modify) the most dangerous microorganisms is presented in Figure 3 [Gronvall et al., 2007, Gronvall, 2008]. The figure does not include potential BSL-4 (or equivalent) military facilities suspected of operating at secure locations in some countries (especially in Asia and the Middle East).

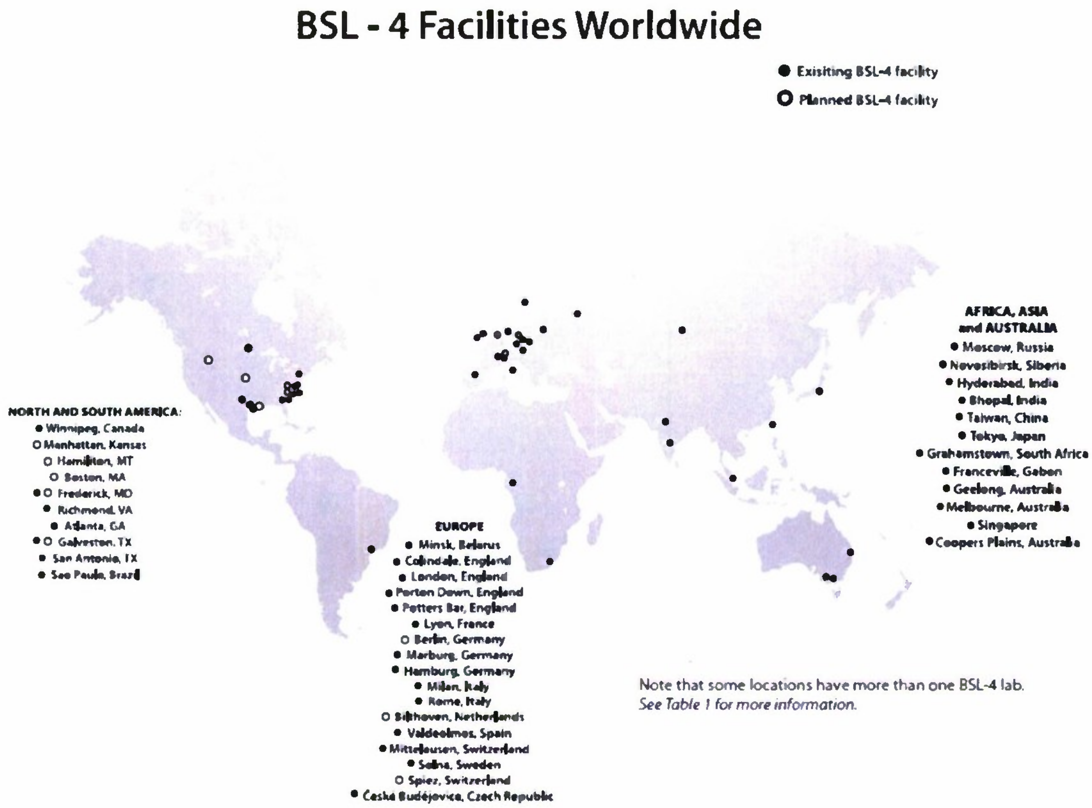
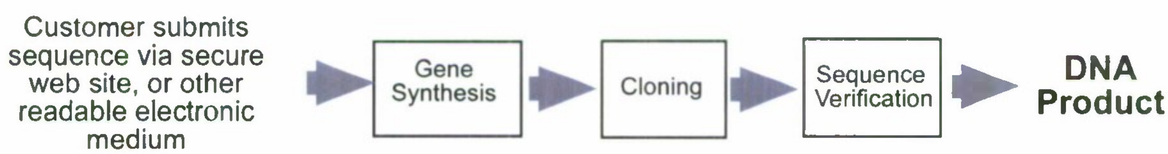


Figure 3. High Containment Laboratories Worldwide
(reproduced with permission from Gronvall, 2008)

Although still important, the monopoly of large laboratories to produce GEOs has declined with the advent of service laboratories and access to information.

Commercial service facilities can do the work for a fee. DNA methodology (e.g., synthesizers and amplification by polymerase chain reaction) has been available and used in academic research for decades. However, especially during the last decade, there was an expansion of private companies providing DNA synthesis and amplification services. These companies are generally called "DNA foundries." However, what makes these companies of particular interest here is their business model and mode of operation (summarized in Figure 4) that result in quick and efficient chemical synthesis of DNA and modification of organisms on demand. After receiving a request for synthesis of a DNA fragment, the foundry does not need to scrutinize the genetic code requested to process and fill the order. Whereas, a survey of DNA foundries (primarily in the United States and Germany) indicated that many do review requests for potential pathogens; many companies neither expend the effort nor possess the specific expertise in performing bioinformatics analysis. Because other countries may not have legal provisions comparable to those (select agent list) of the United States, diligence to scrutinize the nucleic acid being synthesized can not be globally assumed. In addition, several technical advances could allow masking of the target organism being pursued (see Section 5) even if screening controls were in place.



The essence of the foundry process is that DNA can be synthesized solely from the sequence provided in electonic format.

Figure 4. DNA Foundry Process

The significance of this commercial capability is that a customer with web access can obtain (for a price) complete synthetic genes of any organism whose genome is known or even complete synthetic viruses. Thus, a customer can gain access to genes of a threat organism (or even complete viruses) without possessing the necessary laboratory infrastructure that is supplied "on demand" by the DNA foundries.

Although DNA foundries vary in company size and in their duration in activity, we investigated the DNA services more easily available. The cost estimates obtained during October 2007 ranged between $1,800 to-$2,500 per 1000 bases. For comparison, the whole genome size of the Ebola virus is approximately 19,000 bases long [Volchkov et al., 2001]. The geographical distribution of DNA foundries available during July 2005 and depicted in Figure 5 demonstrates that genetic synthesis is a global capability very difficult to monitor by any single country.

It is unlikely that the DNA foundry will be able to provide the complete microbial genome in a single construct. Moreover, it will be considerably difficult to assemble the purchased pieces into a viable infectious organism. Considerable knowledge to properly design threat agents, to assemble the purchased synthetic segments, and to end with an organism that can propagate in cell culture and animals is still necessary and not trivial. However, the capability to purchase sequences encompassing whole organisms (particularly viruses, which are relatively small) or genes to modify and enhance pathogenic organisms (viral or bacterial) arguably exists in the free market.

Although whole synthesis is relatively easy to envision and to achieve by using commercial suppliers, the modification of available microorganisms by a variety of novel approaches could be a totally in-house alternative to synthesis *de novo*.

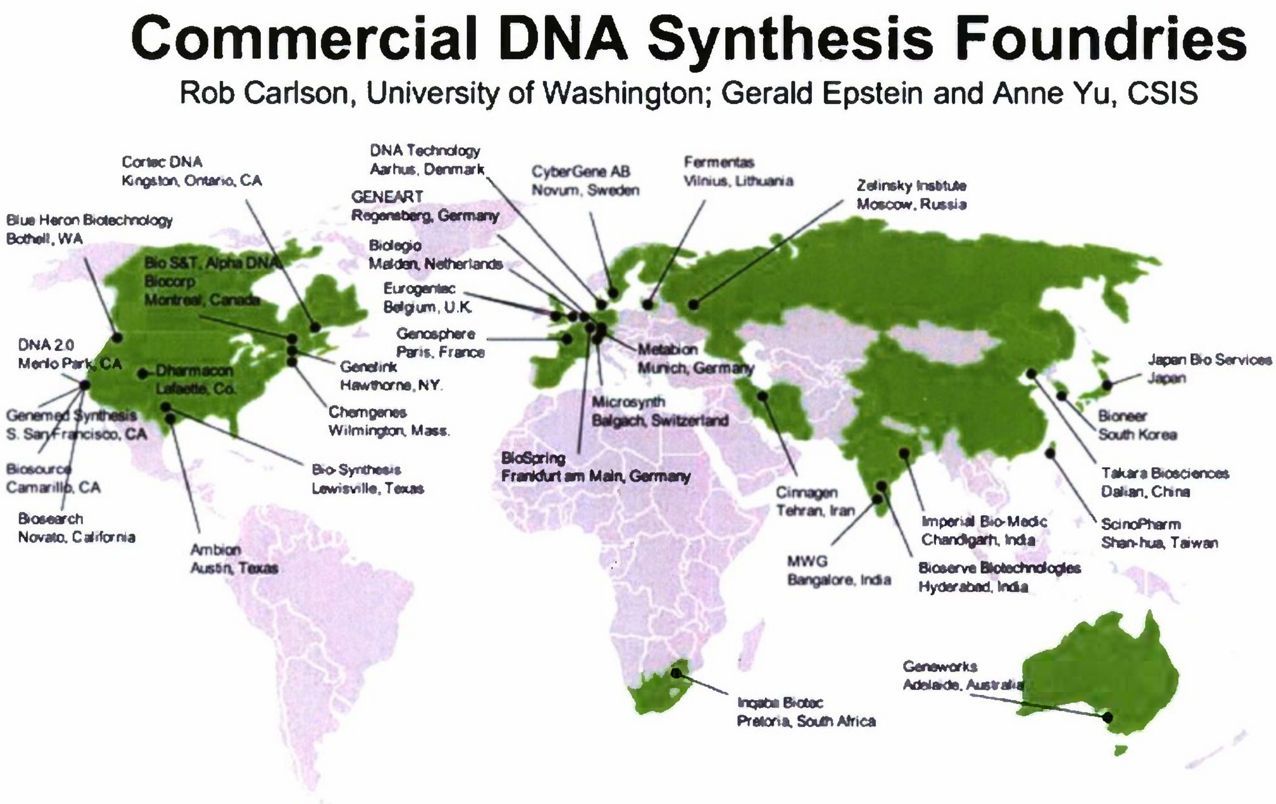


Figure 5. Global Distribution of DNA Foundry Service

Data source: Carlson R. (2003) The pace and proliferation of biological technologies. Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. *1 (3)* 1-19 (www.kurzweilai.net/articles/art0614.html?) accessed September 2007. Also reproduced in presentation by Working Group on Synthetic Genomics: Progress Report, National Science Advisory Board for Biosecurity (NSABB) Meeting, March 30, 2006.
Reproduced with permission from Dr. R. Carlson, November 2008.

In addition to standard *in vitro* cloning techniques or chemical synthesis *de novo*, there is a variety of either alternative or complementary approaches which, due to their efficiency and simplicity, may be used to produce GEOs. These approaches involving *in vivo* recombination of desired sequences or properties include: DNA shuffling or molecular breeding [Stemmer W.P.C., 1994; Soong et al., 2000], recombineering [Court et al., 2002; Thomason et al., 2007], and genomic reconstruction in yeast artificial chromosomes [Markie et al., 2006]. The reported total times involved in some of these methods range from approximately a week to 10 days. These techniques have been used to produce DNA molecules that were too large to manipulate with classical techniques and for generating a chimerical virus that was infectious in a new (previously resistant) mammalian host.

Therefore, even if some day the orders for DNA synthesis received by DNA foundries could be either watched or regulated, alternative technologies would allow the dedicated scientist to pursue GEO development by means other than *de novo* synthesis.

7. SMALL INVESTMENT AFFORDS CONSIDERABLE CAPABILITIES

Once desired genes have been synthesized (or obtained by other approaches) and inserted into the host organism (by standard recombinant DNA or alternative techniques), the engineered organism is then amplified. The efficiency of DNA recombination does not need to be high because the newly modified agents are selected to be at least as self-replicating and infectious as their starting parents. Thus, only a very small number of engineered organisms are required as seed to expand them into production. In addition, relatively small quantities of GEOs may be sufficient to pose a significant threat. The portion of in-house work required to recombine DNA, and select and amplify GEOs requires a relatively low investment, particularly given that most of the equipment is widely available for purchase on second-hand markets (including e-bay!). Table 2 provides the cost of equipment needed for a basic and relatively small operation involved in producing GEOs. This represents an effort heavily dependent on DNA service laboratories and foundries with limited production output. Yet, such production is still capable of seriously threatening national security. The cost for equipment needed in a more comprehensive operation with enhanced capabilities of DNA sequence, DNA synthesis, and work with viruses is also shown in Table 2.

These costs assume that existing laboratory facilities are used. The total infrastructure costs shown in Table 2 should be increased (perhaps by a factor varying between 3 to 10) to account for additional expenses that may be associated with modifying an existing facility or building structure to accommodate a clandestine operation. Although the costs of weaponization are not included, the costs included in Table 2 demonstrate that investment in a biotechnology facility required for production of GEOs is relatively small when compared to the potentially disruptive effect on public health, social activities, and the economic impact of producing and releasing genetically enhanced threat agents. The current market prices indicate that all of the necessary equipment and supplies for production of small quantities of genetically modified infectious agents can be obtained for the cost of a single luxury car.

Table 2. Small Facility Equipment Cost

Sources: American Lyophilizer, Inc., http://www.freezdrying.com; Cole-Parmer, http://www.coleparmer.com/catalog/; New England Biolabs (Reagents and Supplies), http://www.neb.com/nebecomm/products/; ebay (used equipment)

Capability	Basic	Enhanced
Gene Sequencer (Refurbished 48 Capillary ABi 373)		$ 25,000
GeneSynthesizer (Polyplex 96-well plate high speed synthesizer)	$ 4,000	
ABi 392 DNA/RNA synthesizer/96 Well High Speed		$ 65,000
PCR Synthesizer		$ 1,500
Fermentor/bioreactor	$ 5,000	
Automated controller for Bioreactor		$ 8,500
High Quality Glove Boxes (2)	$ 4,000	$ 6,000
Co2 Incubator (Basic for Viruses)	$ 6,000	$ 19,000
Cell factory or roller bottles, Basic for viruses	$ 3,000	$ 24,000
Dryer/lyophilizer (Laboratory size <15 L capacity)	$15,000	$ 45,000
Refrigerator/Freezer (Large)	$ 3,000	$ 15,000
General laboratory equipment, pH meter, centrifuge, balance, temperature controlled water baths, etc.	$10,000	$ 10,000
Reagents, restriction enzymes, expendable supplies, etc.	$10,000	$ 15,000
Total	$60,000	$234,000

8. GLOBAL INFRASTRUCTURE RELATED TO ENGINEERING INFECTIOUS AGENTS

There is public health infrastructure in most parts of the world, and all but the poorest countries have some capability for research in infectious diseases. Most major academic and national governments have departments and ongoing activities in the technology. (Appendix B includes a small sampling (by country) of capabilities in infectious diseases.) Over this global background of capabilities in infectious diseases, we used the following five indicators to estimate the potential capability of nations to undergo GEO development:

a. The Military Critical Technologies List (MCTL) consensus regarding World Technology Capabilities [Department of Defense, 2007].

b. Existence of a strong academic research infrastructure in infectious disease, as documented by relevant publications. Capability was derived from searches on PubMed (databank of biological research publications [www.ncbi/nlm/gov/sites/entrez], which were subsequently filtered and categorized by various criteria). The statistical results are summarized in Section 9.

c. Maintenance of facilities for handling virulent diseases, specifically, Bio Safety Level 4 (BSL 4) laboratories (Appendix B). Although these facilities are a valuable indicator of national capability and interest, such biocontainment facilities would not be required for small-scale/clandestine operations or in a program where relatively high risks could be tolerated.

d. Existence of commercially viable DNA foundries, which provide evidence of demand, indicating a substantial level of activity.

e. Maintenance of a world-class gene data base accepted as authoritative.

Pertinent statistics were compiled, and a qualitative score from 0-4 was assigned to the countries based on their respective standing in each of the indicators. Data to score indicators "c" and "e" were obtained mainly from searching information posted on the World Wide Web, and the names of a few high containment facilities maintained in several countries are listed in Appendix B. Indicator "d" was scored using information presented in Figure 4. The research infrastructure (indicator "b") was estimated from the total number of publications that related to infectious genetically modified organisms in PubMed searches with the limitation "humans" selected. The composite list of PubMed citations comprised over 8,000 articles, of which over 5,000 were from foreign authors. Select subsets of publications from the last 5 years were used as the basis for scoring countries' research infrastructure. Indicators "a" and "b" were weighed more heavily (1.5 X) than the others because the publications were specific to the subject, and the MCTL evaluation of capabilities represents expert consensus of an interagency technology working group. The results of this qualitative analysis are captured on the map presented in Figure 6.

As shown on the map in Figure 6, significant capabilities were noted on every continent. In addition, a relative absence of capability may have failed to reveal clinical sampling of virulent diseases, small scale research, or capabilities migrating from outside national boundaries with ease. This analysis should not be construed as an evaluation of any specific country (more specific analysis is attempted in Section 9); instead, the analysis should serve as evidence that technology and resources to manipulate and engineer infectious organisms are widely available.

There are difficulties in analyzing this global distribution of capabilities due to the coexistence of research in genetic engineering of organisms for applications in biomedicine, agriculture, and biotechnology with well established research in biological threat agents, and a flood of new scientists in biodefense. The funding available after September 11, 2001, particularly in the United States after the attacks with anthrax in the U.S. postal service, has spun numerous new centers associated with biodefense and feverish activity among government contractors. There were only two BSL-4 laboratories in the United States before 1990. This number increased to three between 1990 and 2000. Since 2001, the number of BSL-4 laboratories in the United States rose from 3 to 15 by 2007 [U.S. General Accountability Office, 2007].

However, expertise can not be created as fast or as easily as funds can be made available or buildings constructed. For example, 439 principal investigators (PIs) received research grants between 2001 and 2005 from the National Institutes of Health (NIH) in Allergy and Infectious Diseases to work on anthrax, brucellosis, glanders, plague, melioidosis, and tularemnia. Only 15 PIs received funding for research with those threat agents before 2001 [National Institute of Allergy and Infectious Diseases (NIAID), Biological Weapons Agent

Funding and Public Health, 2006]. The flood of relatively inexperienced scientists (mainly in academia and private contracting companies) attempting to work with deadly biological agents raises the risk for any one of them to either willingly or unwillingly introduce genetic changes in pathogenic microbial genomes of unprecedented consequences (e.g., the Australian mouse experiment discussed in Section 6). Thus, it should be expected that highlighted attention (following funding) in biodefense could foster, even by chance, at least some project resulting in novel biological warfare capabilities. This has already happened as evidenced by some of the research presented in Section 6.

Figure 6. Global Perspective

9. SCIENTIFIC OUTPUT RELATED TO GENETIC ENGINEERING IN VARIOUS COUNTRIES

In previous sections, we showed that the massive amount of information contained in microbial genomes is freely available; that limited capital investment is needed to manipulate organisms; and that synthetic and research infrastructures to generate infectious organisms from freely available sequences are widespread throughout the world. Thus, the major obstacles to producing GEOs should be neither money nor a source of infectious agent as

starting material. The key to successful GEOs is knowledge (on how to use the available information to design a viable engineered organism that performs as desired).

In this section, we will attempt to investigate how the available capabilities are capitalized by different countries. We made the following assumptions:

a. The best estimate of scientific capabilities is research productivity.

b. The best estimates of research productivity are articles published in technical journals after review and evaluation by subject matter experts.

c. Although covert activities in offensive genetic engineering likely will never be disclosed in the open literature, preventing scientists from interacting with the scientific community and publishing basic research findings degrades expertise in genetic engineering rather quickly. We would even suggest that the most certain measure to quickly loose any offensive capability in biological warfare would be for either a group or country to isolate its bio-scientists.

d. Therefore, we assume that genetic engineering for offensive purposes should always be associated somehow to legitimate research, albeit the link between the two activities may be difficult to establish.

All these assumptions can be summarized as follows:

Any offensive program involving genetically engineered organisms must have rather sophisticated scientific talent, which would be quickly lost (in terms of just a few years) if not continuously challenged and reevaluated by peers in the scientific community. Accordingly, we evaluated the scientific output in the open literature (noting the reported addresses of the authors) that related to capabilities in genetic engineering of microorganisms of interest in biodefense. Selection of key words for our search was not easy because a number of surrogate terms had to be selected. We selected keywords that accounted for research in specific threat agents, methodology that is key to genetic engineering, and research related to human infection and virulence. After extensive discussion and consultations, our search terms included: modified genome virus, Pox virus gene, hemorrhagic virus humans, Ebola, Filovirus, anthracis modified, gene sequence anthracis, modified gene sequence human, recombinant infectious human, and transgenic infectious human. These key words were not intended to be all inclusive, to assure that every research related to GEOs would be detected, or that research groups with potentially malignant goals would be identified. More modestly, these keywords were intended to determine whether rather generic keywords would still be able to relate to temporal and geographical trends in technical capabilities related to GEOs in various countries.

We researched PubMed's total holdings between 1997-2007. PubMed is the leading database of publications in biomedical literature made available through the NCBI at the National Library of Medicine (NLM), located at NIH [www.ncbi/nlm/gov/sites/entrez, accessed in September 2007]. Bibliographic searches with individual keywords produced different numbers of publications by various countries. However, by merging searches with different

keywords, the relative ranking of countries converged to become relatively constant. The search results corresponding to articles associated with all the keywords listed in the previous paragraph were fused, and duplicated articles retrieved by more than one keyword were removed from the list. Although U.S. authors and institutions are heavily weighed in PubMed data, even the relatively narrowly defined search keywords used returned a relatively large number of results from several countries, adequately supporting analysis of global trends. The results obtained are depicted in Table 3 where countries are listed according to the decreasing number of publications (during 2002-2007) associated with the keywords listed above.

The data in Table 3 illustrate the contrast between the considerable lead of the United States in research output and the many countries of the world with very modest scientific impact in areas of interest. Data also show that the number of publications reported by each country in areas related to our selected keywords have increased significantly between 2001 and 2007. The assessment was not exhaustive, in that other combinations of related key words might have been considered. However, the terms used were selected to provide the largest sample of relevant results. Thus, it is considered unlikely that the relative order or the trend would change by selecting different keywords related to genetic modification and infectious organisms. For each country, we calculated the relative increase (as percentage) in the number of publications produced between 2002 and 2007 to those published between 1997 and 2001 and presented the results in Table 4. As would be logically expected, countries with relatively large output were more consistent among the periods of study than those with occasional publications in the queried subjects. To account for differences in the absolute number of research publications, Table 4 presents the countries with more than 20 related publications and a considerable increase in related scientific productivity grouped as (I). Those countries with a considerable increase in related scientific productivity, but based only on a number of related publications between 10 and 19 grouped as (II), and those countries with a large productivity (over 20 publications) but with a modest increase grouped as (III). Table 4 also includes countries with a considerable number of publications in the most recent period studied grouped as (IV); however, no output is recorded in the period between 1997 and 2001 (therefore, no percentage increase could be calculated). The data shown in Table 4 indicate that the increase in research output in the countries with extensive productivity and capabilities in genetic engineering (in group III) was relatively modest (<50% between the two studied periods within the last decade). Similarly, modest increases (or relatively constant levels) were observed in many other countries (from Table 3). In contrast, the scientific publications retrieved by keywords related to microbiology and genetic engineering rose from 80% to several-fold between 2001 and 2007 in several countries (Table 4).

Table 3. Related Scientific Output by Various Countries

Countries	97-01	02-07	Countries	97-01	02-07
United States	1402	2068	Malaysia	3	6
Japan	217	390	Slovenia	1	6
Germany	235	299	Chile	4	5
U. Kingdom	210	281	Ukraine	4	5
China	75	281	Costa Rica		4
France	162	213	Uganda	1	4
Canada	94	179	Saudi Arabia	1	3
Italy	68	115	Nepal		3
Spain	69	114	Kenya	2	3
Australia	61	88	Hong Kong	3	3
Netherlands	62	79	Tunisia	1	3
Sweden	55	76	Sri Lanka		2
Switzerland	44	63	Serbia	3	2
Brazil	16	59	Senegal	2	2
South Korea	19	52	Portugal	2	2
Taiwan	19	40	U. Arab Emirat.	1	2
India	25	40	Philippines	1	2
Belgium	18	40	Luxembourg		2
Denmark	16	34	Congo ROC		2
Thailand	16	33	Bangladesh		2
Israel	17	33	Nigeria		2
Russia	31	32	Trinid Tobago		1
Finland	17	29	Slovakia	1	1
Austria	24	27	Rwanda		1
Turkey	3	25	Peru	1	1
Singapore	4	22	Ivory Coast	3	1
Cuba	11	22	Georgia		1
South Africa	5	18	Gambia		1
Poland	11	18	Dominican R		1
Czech Rep	9	18	C.African R.	3	1
Norway	14	15	Cambodia		1
New Zealand	6	14	Burkina-Faso	1	1
Greece	6	14	Bulgaria	1	1
Mexico	8	13	Barbados	1	1
Argentina	15	13	D.R. Congo	7	
Iran		12	Ukraine	4	
Gabon	6	12	Kuwait	3	
Vietnam	4	11	Bolivia	1	
Ireland	5	10	Oman	1	
Hungary	7	10	Nicaragua	1	
			Myanmar	1	
Colombia		8	Libya	1	
Croatia	3	8	Latvia	1	
Pakistan	2	7	Sudan	1	
Indonesia	4	7	Tanzania	1	
Venezuela	3	7	Zimbabwe	1	

Table 4. Variation in Related Research Output between 2002-2007 and 1997-2001

Group	Country[1]	Increase[2]	Capabilities[3,4]
(I) Pub >20 (2001-2007) Increase >80%	China	275 %	C, DF[5]
	Brazil	269 %	C, G-4
	South Korea	174 %	C, DF
	Belgium	122 %	C, DF
	Denmark	112 %	C, DF
	Taiwan	110 %	C, DF, G-4, MIL-4
	Thailand	106 %	C, (U.S. Arm. Forces I., CDC)
	Cuba	100 %	C[6]
	Israel	94 %	C[7]
	Canada	90 %	C, DF, G-4
	Japan	80 %	C, DF, G-4
(II) 10<Pub<19 Increase >80%	Turkey	733 %	C[8]
	Singapore	450 %	C, G-4
	Vietnam	150 %	C (Pasteur Inst.)
	Greece	133 %	C
	New Zealand	133 %	C
	Czech Rep	100 %	C, G-4
	Gabon	100 %	G-4 (Pasteur Inst.)
	Ireland	100 %	C
(III) Pub >20 Increase < 50%	United States	47 %	C, DF, A-4, G-4, MIL-4
	U. Kingdom	34 %	C, DF, G-4, MIL-4
	Germany	27 %	C, DF, A-4
	Russia	3 %	C, DF, G-4, MIL-4
(IV) Pub >3 in (2002-2007) No pub before	Iran	0-12[1]	C, DF (Pasteur Inst.)
	Colombia	0-8	C
	Costa Rica	0-4	C

1. Grouping according to number and relative increase of publications between 2007-2002 and 1997-2001 from Table 3.
2. Relative increase calculated as {[Number of related publications between 2002-2007 (from Table 3) divided by the number of publications during 1997-2001] - 1) x 100}
3. Known capabilities are noted:
   DF - DNA foundries able to synthesize DNA on demand (Section 6)
   C - Advanced research capabilities as indicated by well established laboratories and by key scientific findings reported in the literature (Section 7)
   Mil - Military microbiological capabilities (some listed in Appendix B)
4. High containment laboratories able to operate with BSL-4 (most dangerous) pathogens. (Some listed in Appendix B) P-4 or A-4 indicates private or academic BSL-4 facilities, respectively. G-4 inidicates a Government BSL-4 facility. Mil-4 indicates existence of at least one BSL-4 laboratory under military control (Appendix B)

5. China had at least 500 enterprises associated with life science and biotechnology, with 50,000 employees in 2004 [Qian W. (2005). Efforts to strengthen biosafety and biosecurity in China http://cns.miis.edu/pubs/week/pdf_support/070917_wang.pdf (accessed October 2008)]. A biosecurity program exists, particularly associated with the Academy of Military Sciences; but, activities with BSL-4 pathogens are clouded by multiple layers of bureaucracy.
6. Cuba has sophisticated capabilities, particularly in virology. Biological military activities have been claimed by a Cuban defector in Florida; but, accusations have not been confirmed by other sources [www.upmc-biosecurity.org/website/biosecurity_briefing/bb_index_archive/2007-03-02-index.html#bb3, August 2008].
7. The Israel Institute of Biological Research in Ness Ziona has capabilities in infectious organisms, engineering of protein and enzymes and in functional genomics [www.iibr.gov.il, August 2008]. Funding by the Israel Ministry of Defense is acknowledged; but, projects are not described.
8. The Gulhane Military Medical Academy (Ankara) and the NucBiolChem School of the Turkish Armed Forces (Kucukyali, Istanbul) appear to work on advanced biodefense projects; but, specific information is lacking.

Examples of advanced capabilities that are offshoots from parent laboratories in foreign countries (United States and France) are presented in parenthesis.

Laboratory facilities associated with historically relevant biological military capabilities are generally known in countries having high scientific output. Several countries identified in Table 3 as having increased their activities related to GEO technology by two-fold or more also have facilities (see Table 4) that could be potentially directed to GEO activities of military relevance.

The increase in related scientific output could be potentially misleading if not related to some index reflecting the overall activity of each country. We investigated whether the increases detected in research activity could be related to a general national expansion and growth. Comparison between relative increases in the scientific output in the technical subjects that we searched and the National Gross Domestic Product (GDP) is presented in Table 5. Ireland that developed extensive biotechnology capabilities during the period that we studied shows that the 100% increase observed in related scientific output (Tables 3 and 4) directly correlates to the national economic growth (119%). Similarly, the high increase in scientific output of several countries listed in group (I) of Table 4 (Belgium, Denmark, Thailand, and Canada) can be explained by the increase in their respective domestic growth. Countries with highly developed biological capabilities (and a history of previous activities in biological warfare, group III at the bottom of Table 3) had a modest increase in scientific output compared to growth of their respective GDP during the period studied (Table 5). The relative increase in technical output of some countries was lower (Taiwan, Cuba) or much lower (Germany, UK, France, Russia) than expected from their respective GDP (Table 5). Yet, the increase in research activity related to genetic engineering of microorganisms far outpaced (2-20 fold) the growth in the domestic economy of five and two countries in groups I and II, respectively (Table 5).

Genetic engineering is a key enabling technology for a wide spectrum of socially desirable ends. Therefore, the results presented in Table 5 should not, in any way, be construed as suggesting that countries with GEO related activity that is disproportionately higher than their economical expansion are more likely to engage in genetic engineering for malicious purposes (including offensive weapons). The results in Table 5 only indicate that the scientific interest and output on areas related to genetic engineering has substantially outpaced the overall increase of the respective national economy in some countries.

Table 5. Increase in Research Output Related to Genetic Engineering Referred to National Economic Expansion

Countries [1]	Δ Science[2] / Δ GDP	GDP Increase[3] (Δ)	GDP 2006 (Million U.S. Dollars)[4]	GDP 2001 (Million U.S. Dollars)	Δ Science[5] 2006-2002/ 1997-2001
Japan	20 X	3.9%	4,340	4,176	80%
Israel	11 X	8.8%	123	113	94%
China[6]	2.8 X	98%	2,668	1,345	275%
Brazil	2.4 X	110%	1,068	508	269%
S. Korea	2.1 X	84%	888	482	174%
Belgium	1.7 X	72%	392	228	122%
Denmark	1.5 X	73%	275	159	112%
Thailand	1.4 X	78%	206	116	106%
Canada	1.1 X	80%	1,251	694	90%
Taiwan[7]	0.8 X	133%	682	292	110%
Cuba[7]	0.7 X	142%	46	19	100%
Singapore	8.2 X	55%	132	85	450%
Turkey	4.1 X	180%	403	144	733%
Vietnam	1.8 X	85%	61	33	150%
Ireland	0.8 X	119%	223	102	100%
U.S.A.	1.5 X	32%	13.202	10.020	47%
U.K.	0.53 X	64%	2345	1,430	34%
Germany	0.47 X	57%	2,907	1853	27%
France	0.45 X	69%	2,231	1,320	31%
Russia	0.014 X	221%	987	307	3%

1. The top two groups include countries with an increase of 80% or higher in scientific output (2002-2007/1997-2001) and either (I) over 20 related publications in the 2002-2007 period [top] or (II) between 10 and 19 related publications [middle]. The group (III) at the bottom includes countries with output larger than 20 related publications, but with a scientific output increase smaller than 50%.
2. Calculated as relative increase (%) in scientific output (2002-2007/1997-2001) divided by relative increase (%) in GDP (2006/2001)
3. Calculated as [(GDP in 2006 divided by GDP in 2001)-1] x 100
4. The GDP was obtained from the years indicated from the World Bank Indicators Database, World Bank ,1 July 2007, www/site.resources.worldbank.org/DATASTATISTICS/Resources?GDP.pdf; http://en.wikipedia.org/wiki/List_of_countries_by_past_GDP_(nominal) (accessed December 2007).
5. ΔScience corresponds to the increase in publications associated with the queried keywords and published during 2002 and 2007 divided by the number of publications between 1997 and 2001 (x 100%) from Table 3.
6. Does not include Hong Kong
7. Estimated GDP from www.workmall.com/wfb2001/cuba/cuba_economy.html; www.cia.gov/library/publications/the-world-fact-book/geos/tw.html#Econ; www.cia.gov/library/publications/the-world-fact-book/print/cu.html; (accessed December 2007)

In other words, the data do suggest that a technical capability that could be potentially related to offensive purposes using genetic engineering was developed either by chance or by national decision at a higher pace in some countries than their respective GDP would have suggested.

10. CONCLUSIONS

All nations need to face the reality that capabilities to develop and produce genetically engineered organisms (GEOs), particularly viruses, are readily available at costs that make them within the reach of even the most impoverished nation, as well as within the grasp of many small groups or individuals.

The principles to GEOs described in the first sections of this report are within the grasp of advanced college or graduate students. The basic information needed to synthesize genetic material is widely-shared and disseminated in databases freely accessible on the Internet. The commercial facilities that can synthesize microbial genomes on demand are globally distributed. Current DNA foundries have the capability to produce sequences of as many as 40,000 bases/base pairs. This is more than adequate to completely synthesize many viruses. Novel approaches indicated in Sections 2 and 4 could allow preparation of novel organisms totally in-house or by purchasing oligonucleotides that can not be easily correlated to the targeted threat agent. A working facility for assembling GEOs can be readily purchased for a modest price. Therefore, the only limitation to producing GEOs for licit or offensive purposes is knowledge on the biology and structure of viruses and bacteria, practical use of available genetic information, and mastery of more complex and sophisticated molecular manipulations to successfully produce an infectious engineered agent.

References in the scientific literature demonstrate that viruses having no counterpart in nature have been synthesized already (Section 4). They have also shown that viruses have been engineered to gain a new mammalian host, which was not infected by the natural virus. Manipulating genes even for licit purposes may have unexpected adverse consequences [e.g., those seen in the 2001 Australian experiment (Section 2 and 4)]. A combination of the scientific accomplishments listed in Section 4 indicates that engineered organisms might jump species and/or result in diseases against which large populations of plants and animals have little or no immunity or resistance.

The scientific attention and productivity on scientific research related to enabling technologies of relevance to genetic engineering has steadily increased in many countries. In some countries, these activities have outpaced the respective economic growth. Several of the countries where GEO related activities outpaced their respective Gross Domestic Products (GDPs, Table 5) have facilities associated with the military (Table 4).

Although an argument can be made that the probability of producing an agent effective for biological warfare is relatively low, the potential impact of an adversary succeeding in such an endeavor is incalculable. The potential threat of GEOs to national security is further compounded by the difficulty faced by governments to develop adequate countermeasures. A

regulatory government response to the risk of a biological attack (e.g., enforcing the Select Agent Rules) is unlikely to prevent members of a warfare program from acquiring threat agents by either (I) obtaining samples during outbreaks of emerging and re-emerging diseases, particularly those occurring in parts of the world were government has little control or (II) using current technologies in microbial genetics that allow producing specific portions or total genomes of infectious agents without ever accessing the agent.

The funding available after September 11, 2001, particularly after the attacks with anthrax in the U.S. Postal Service, has spun numerous new centers associated with Biodefense. The vast majority of newly formed Centers and Institutes for Biodefense are little more than academic exercises to obtain funding. However, the attention in biological warfare fostered some research projects, like synthesis *de novo* of dangerous viruses and other advances (summarized in Section 4) that could facilitate or at least inspire the pursuit of biological warfare capabilities. We share the opinion that as a result of increased interest and technical advancement, the global risk for an attack with GEOs of unpredictable consequence is higher today (2008) than it was in 2001.

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APPENDIX A

CDC CLASSIFICATION OF "SELECT AGENTS"

The CDC has classified "select agents" (http://www.cdc.gov/nasd/docs/d001701-d001800/d001780/d001780.html (accessed September 2007) according to the degree of danger each agent is felt to pose into one of the following three categories:

Category A - Biological Disease:

High-priority agents include organisms that pose a risk to national security because they

- can be easily disseminated or transmitted person-to-person
- cause high mortality, with potential for major public health impact
- might cause public panic and social disruption
- require special action for public health preparedness

These agents/diseases include:

- Bacillus anthracis (anthrax)
- Clostridium botulinum toxin (botulism)
- Yersinia pestis (the plague)
- Variola major (smallpox)
- Tularemia (Francisella tularensis)
- Hemorrhagic fever due to:
    - Ebola virus
    - Marburg virus

Category B - Biological Disease:

Second highest priority agents include those that

- are moderately easy to disseminate
- cause moderate morbidity and low mortality
- require specific enhancements of CDCs diagnostic capacity and enhanced disease surveillance.

These agents/diseases include the following:

- Q fever (Coxiella burnetii)
- Brucellosis (undulant fever)
- Glanders (Burkholderia mallei)
- Ricin toxin (from the castor bean Ricinus communis)
- Epsilon toxin of Clostridium perfringens (the gas gangrene bacillus)
- Staphylococcus enterotoxin B (staph toxin)

Category C - Biological Disease:

The third highest priority agents include emerging pathogens that could be engineered for mass dissemination in the future because of

- availability
- ease of production and dissemination
- potential for high morbidity and mortality and major health impact

These agents/diseases include:

- Nipah virus
- Hantavirus (the Hantavirus pulmonary syndrome)
- The tickborne hemorrhagic fever viruses
- The tickborne encephalitis viruses
- Yellow fever
- Tuberculosis (multidrug-resistant TB)

APPENDIX B

RESEARCH CENTERS IN VARIOUS COUNTRIES

A listing of Biosafety Level 4 (BSL 4) Facilities (**in Bold**) that can work with the most dangerous biological agents was obtained from the Interpol database (update of 27/3/2006, http://www.interpol.int/Public/BioTerrorism/links/biocontainmentLab.asp; Wikipedia: Biosafety Level (accessed July 2007), and from The Sunshine-project (www.sunshine-project.org/biodefense/ (accessed August 2008)

Argentina:

- Structural Bioinformatics Group - Structural Bioinformatics Group at Quilmes National University in Argentina
- National Institute of Infectious Diseases, Buenos Aires

Australia:

- **Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australian Animal Health Laboratory (AAHL), BSL-4,** Geelong, Australia http://www.csiro.au/csiro/content/standard/pps84,,.html
- **National High Security Quarantine Laboratory (NHSQL) of the Victoria Infectious Diseases Reference Laboratory, BSL-4**, Melbourne, Australia
- **Virology Laboratory of the Queensland Department of Health, BSL-4**, Queensland, Australia
- Australian Research Council in Bioinformatics
- Institute of Molecular Bioscience, University of Queensland
- Monash University (Part of the Victorian Bioinformatics Consortium)
- Virus Databases at the Australian National University's Bioinformatics Facility from the Research School of Biological Sciences. At this site, one can expect to find nomenclature, characterization, and general information about viruses throughout the world. There are also genome searches and electron micrographs.
- Infectious Diseases Laboratories, Institute of Medical and Veterinary Science, Adelaide richard.lumb@imvs.sa.gov.au
- Centre for Infectious Diseases and Microbiology, Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead, New South Wales

- Institute for the Biotechnology of Infectious Diseases, University of Technology, Sydney, New South Wales
- Infectious Diseases and Immunology Division, Queensland Institute of Medical Research and The University of Queensland

Austria:

- Department of Internal Medicine, University of Vienna Medical School (reported work in Dengue hemorrhagic fever)
- Innsbruck Medical University (reported work in pox viruses)
- Vienna General Hospital (reported work with an anthrax disinfectant)

Belgium:

- Department of Microbiology and Department of Clinical Sciences, Institute of Tropical Medicine Antwerp ebottieau@itg.be
- Department of Microbiology and Immunology, Infectious Diseases Research Group, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven johan.vaneldere@rega.kuleuven.ac.be

Brazil:

- **Fundação Oswaldo Cruz, under the Brazilian government**, **BSL-4,** Rio de Janeiro, Brazil
- Functional Genomics and Bioinformatics, at the Department of Biochemistry and Molecular Biology (DBBM), Oswaldo Cruz Institute (IOC), FIOCRUZ, Rio de Janeiro
- Laboratory of Bioinformatics (University of Campinas)
- Bioinformatics Laboratory (Catholic University of Brazil)
- Division of Infectious Diseases, Universidade Federal de São Paulo, SP 04023-062
- Institute of Infectious Diseases, Emilio Ribas, Sao Paulo
- Laboratory of Virology (LIM-52) - Department of Infectious Diseases, School of Medicine and Tropical Medicine Institute (IMT), University of São Paulo, São Paulo pierrot@usp.br

Bulgaria:

- Department of Infectious Diseases, Epidemiology and Parasitology and Department of Microbiology and Immunology Higher Medical Institute, Plovdiv

Canada:

- **National Microbiology Laboratory, Canadian Science Centre for Human and Animal Health, BSL-4**, Winnipeg, Canadahttp://www.nml.ca/english/facilities_capabilities.htm
- University of British Columbia
- University of Waterloo
- University of Victoria (host of a SARS Bioinformatic Web site.)
- Kinexus, which is a for profit concern, "striving to be a world leader in proteomics and bioinformatics for the discovery, development and commercialization of human diagnostics and therapeutics, based on leading-edge knowledge of cell communication proteins."
- Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada, Ottawa ON
- University of Ottawa Hospital, Division of Infectious Diseases, The Ottawa Hospital-General Campus, The Ottawa Health Research Institute, Ontario ccooper@ottawahospital.on.ca
- Faculty of Pharmaceutical Sciences, Health Care and Epidemiology, Division of Infectious Diseases, Department of Medicine, Vancouver Hospital and Health Sciences Centre, University of British Columbia, Vancouver fawziah.marra@bccdc.ca
- Infectious Diseases Control Unit of the Direction de la santé publique, Montreal Chest Institute, Montreal
- Division of Infectious and Immunological Diseases, Department of Pediatrics, University of British Columbia, British Columbia's Research Institute for Child and Family Health, Vancouver

China:

- Center for Bioinformatics (Peking University)
- Institute for Bioinformatics (Tsinghua University)
- Institute of Bioinformatics (Tianjiang University)
- Chinese Academy of Sciences
- State Key Laboratory of Pathogen and Biosecurity, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing
- State Key Laboratory for Infectious Disease Prevention and Control, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 100 YingXinJie, XuanWuQu, Beijing

- Institute of Infectious Diseases and Key Laboratory of Infectious Diseases of Ministry of Health, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou flwnp@yahoo.com.cn
- State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Changping, Beijing
- Institute for Infectious Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing
- Institute of Epidemiology and Microbiology, China National Center for Preventive Medicine, Beijing (reported work in hemorrhagic fevers and anthrax)
- Centre for Emerging Infectious Diseases and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong

Cuba:

- Instituto de Medicina Tropical Pedro Kourí
- Ministry of Public Health of the Republic of Cuba

Czech Republic:

- **Centrum Biologicke Ochrany Techonin** (Cemter of Biologucal Protection), **BSL-4**
- Clinic of Infectious Diseases, University Hospital, Jihlavska 20, CZ-639 00 Brno pchalupa@fnbrno.cz
- The Military Medical Academy and the Faculty of Military Health Sciences of the University of Defense, Hradec Kralove (reported work in anthrax vaccines and Ebola and Marburg viruses)
- Division of Infectious Diseases, Epidemiology Department of Preventive Medicine, Masaryk University in Brno, Joštova 10, 662 44 Brno
- Department of Infectious and Tropical Diseases, Faculty of Medicine, Charles University Prague
- Department of Infectious Diseases, University Hospital, Hradec Králové

Denmark:

- Department of Infectious Diseases Immunology, Statens Serum Institute, Copenhagen S Sho@ssi.dk
- Odense University Hospital (reported work in Denguc fever)
- Department for Infectious Diseases, Rigshospital, Blegdamsvej 3 A, 2200 Copenhagen

Finland:

- Department of Infectious Disease Epidemiology, National Public Health Institute, Helsinki
- Department of Virology, Haartman Institute, POB 21, 00014 University of Helsinki (reported work in hemorrhagic fevers, including Ebola)
- Biological Defence Centre for Biothreat Preparedness

France:

- **Laboratoire Mérieux, BSL-4**, Lyon France. http://www.lab-merieux.fr/us/index.htm
- **Centre International de Recherches Médicales de Franceville (CIRMF), BSL-4**, Gabon. http://www.cirmf.org/ This research organization is supported by the French government in Gabon
- Service des Maladies Infectieuses, Centre Antirabique, CHU de Poitiers, 2, rue de la Milétrie, BP 577, 86021 Poitiers cedex
- Département maladies infectieuses, Institut de Veille Sanitaire, 12 rue du Val-d'Osne, 94415 Saint-Maurice cedex jc.desenclos@invs.sante.fr
- Service de Virologie/UPRES EA 3610, Bâtiment Paul Boulanger, CHRU Université Lille 2, 59037 Lille Cedex
- Infectious Disease Service (P.D.), L'Archet Hospital, Nice
- Information Génomique et Structurale, Institute for Structural Biology and Microbiology, IBSM, Marseille Pierre-Edouard.Fournier@univmed.fr (reported work in emerging infectious diseases)

Germany:

- **Bernhard Nocht Institute (BNI) for tropical medicine, BSL-4**, Hamburg, Germany http://www15.bni-hamburg.de/bni/bni2/neu2/getfile.acgi?area_engl=news&pid=6104
- **Philipps University, BSL-4**, Marburg, Germany http://www.med.uni-marburg.de/stpg/ukm/lt/hygiene/eviro.htm (accessed January 2008)
- **Robert Koch Institute BSL-4**, Berlin (under construction)
- Central Institute of the Bundeswehr Medical Service, Koblenz
- Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin JansenA@rki.de
- Berlin Institute of Tropical Medicine, Berlin
- Division 13, Applied Tropical Medicine and Infectious Diseases Epidemiology, German Navy Institute for Maritime Medicine, D-24119 Kronshagen

- Institute for Molecular Biology of Infectious Diseases, University of Würzburg, D-97070 Würzburg
- Department of Medical Microbiology and Immunology of Infection, Institute of Infectious Diseases Medicine, Benjamin Franklin Medical Center, Free University of Berlin jutta.wagner@medizin.fu-berlin.de
- Institute for Medical Microbiology and Epidemiology of Infectious Diseases, University of Leipzig, Liebigstrasse 24, 04103 Leipzig ackermg@medizin.uni-leipzig.de
- Institute for Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, Ludwig-Maximilian University, Munich

Greece:

- WHO Collaborating Center for Reference and Research on Arboviruses and Haemorrhagic Fever Viruses at Aristotle University of Thessaloniki, Thessaloniki
- Centre of Athens Veterinary Institutes, Institute of Infectious and Parasitic Diseases, 25 Neapoleos Street, 15310 Agia Paraskevi, Attiki
- Department of Parasitology, Entomology and Tropical Diseases, National School of Public Health, 196 Alexandras Ave, 11521, Athens

Hungary:

- Central Institute for Infectious Diseases, Budapest
- Johan Bela National Center for Epidemiology, Budapest (reported work in Dengue fever)

India:

- **High Security Animal Disease Laboratory** (HSADL), **BSL-4**, Bhopal
- India.Department of Gastroenterology, Pushpawati Singhania Research Institute, New Delhi (reported Dengue virus work)
- Infectious Diseases Research Laboratory, Institute of Medical Sciences, Banaras Hindu University, Varanasi drshyamsundar@hotmail.com
- The Kerala State Institute of Virology and Infectious Diseases, Alappuzha, Kerala vlr_tjjohn@sancharnet.in
- Center for Biotechnology at Jawaharlal Nehru University (JNU) and Center for Biochemical Technology, New Delhi (reported work on anthrax vaccine)

Indonesia:

- Laboratory of Infectious Disease, Eijkman Institute for Molecular Biology, Jakarta

- Division of Tropical and Infectious Disease, Department of Internal Medicine Faculty of Medicine, University of Indonesia-dr. Cipto Mangunkusumo Hospital, Jakarta
- National Institute of Health Research and Development, Ministry of Health, Jakarta (reported work in Dengue fevers)

Iran:

- Iran Bioinformatics Center (IBC) is identified as the only academic center in Iran working on Bioinformatics. Another research group is the Bioinformatics and Biomathematics Unit in Mazandaran University of Medical Sciences. The IBC is a part of the Institute of Biochemistry and Biophysics (IBB) in Tehran University and offers a PhD program in bioinformatics.
- Department of Infectious Disease and Clinical Immunology, National Research Institute of Tuberculosis and Lung Disease, Shaheed Beheshti Medical Science University, Tehran
- Infectious Disease and Tropical Medicine Research Center, Shaheed Beheshti University of Medical Sciences, Tehran
- Department of Epidemiology and Biostatistics, School of Public Health, Zahedan University of Medical Sciences, Zahedan (reported work in hemorrhagic fevers)
- Laboratory of Arboviruses and Viral Haemorrhagic Fevers, Pasteur Institute, Tehran

Israel:

- Department of Infectious Diseases, Israel Institute for Biological Research, Ness Ziona lustig@iibr.gov.il
- Infectious Diseases Unit, Assaf Harofeh Medical Center, Zerifin, and the Sackler School of Medicine, Tel Aviv University, Ramat Aviv
- Infectious Disease Unit, and Virology Laboratory, Rambam Medical Center, and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa i_oren@rambam.health.gov.il
- Internal Medicine E and Infectious Diseases Institute, Soroka University Medical Center, Faculty of Health Sciences, Ben-Guion University of the Negev, Beer-Sheva arcohen@clalit.org.il
- Infectious Disease Unit, Bnai Zion Medical Center, Haifa
- The Center for Geographic Medicine and Department of Medicine, The Chaim Sheba Medical Center, Tel Hashomer (reported work in Dengue fever)

Italy:

- **Istituto Nazionale Malattie Infettive, Ospedale Lazzaro Spallanzani**, **BSL-4**, Rome, Italy.http://www.inmi.it/Ita_Home.html
- **Azienda Ospedaliera Ospedale Luigi Sacco-Polo Univresitario BSL-4**, Milano
- Department of Epidemiology, National Institute for Infectious Diseases, IRCCS, Rome
- Institute of Infectious Diseases, Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, 00168 Rome
- Institute of Infectious and Tropical Diseases, University of Brescia, Piazza Spedali Civili 1, 25125 Brescia a.carvalho@libero.it
- Institute of Infectious and Tropical Diseases, University of Milano Fabio.Franzetti@unimi.it
- Department of Infectious, Parasitic, and Immunomediated Diseases, National Institute of Health, Rome
- Institute of Infectious Diseases, University of Bari, Bari p_maggi@yahoo.com
- Division of Infectious Diseases, San Raffaele Scientific Institute, Milan nicola.gianotti@hsr.it
- Institute of Infectious Diseases, Medical School, University "Federico II," Naples
- Institute of Clinical Infectious Diseases, Catholic University, Rome andrea.deluca@rm.unicatt.it
- Institute of Infectious Disease, Department of Clinical and Morphological Research School of Medicine, University of Udine
- Institute of Infectious Diseases, University of Siena
- Institute of Infectious Diseases, Via Massarenti 11, Bologna
- Institute of Infectious Diseases, University of Catania

Japan:

- **Institute of Physical and Chemical Research**, **BSL-4**, Tsukuba, Japan (Listed as non-operational)
- **National Institute for Infectious Diseases, Department of Virology** , **BSL-4**, Tokyo, Japan.http://www.nih.go.jp/niid/welcome/org-index-e.html
- National Institute of Infectious Diseases, Tokyo
- Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, Osaka yoda@iph.pref.osaka.jp
- Department of Infectious Diseases, Tokyo Metropolitan Komagome Hospital

- Division of Tropical Medicine, Kanazawa Medical University, Ishikawa (reported work in Dengue fever)
- Division of Infectious Disease Control and Clinical Immunology, Nihon University Medical Research Institute, 30-1 Ohyaguchi-kamimachi, Itabashiku, Tokyo satoshih@med.nihon-u.ac.jp
- Department of Virology, Faculty of Medicine, University of the Ryukyus, Okinawa (reported work in Dengue fever)
- Department of Infectious Diseases and Applied Immunology, Institute of Medical Science, University of Tokyo
- Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki hamadydieng@hotmail.com (reported work in Dengue virus)

Kazakhstan:

- Kazakh Science Center of Quarantine and Zoonotic Diseases (KSCQZD) (reported work in anthrax)

The Netherlands:

- **National Institute for Public Health and the Environment (RIVM), BSL-4**, Bilthoven Erwin.de.bruin@rivm.nl (under construction)
- Department of Infectious Diseases, Leiden University Medical Centre, Leiden p.j.van_den_broek@lumc.nl
- Eijkman-Winkler Institute for Microbiology, Infectious Diseases and Inflammation, University Medical Center Utrecht, 3508 GA Utrecht A.Paauw@umcutrecht.nl
- Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, Amsterdam cobelensf@kncvtbc.nl

New Zealand:

- Health Research Council of New Zealand's Virus Research Unit, University of Otago, Dunedin (reported work in Dengue fever)
- Infectious Disease Unit, Auckland Hospital

Norway:

- The Division of Infectious Disease Control and the Department of Infectious Diseases Epidemiology, Norwegian Institute of Public Health, Nydalen, NO-0403 Oslo
- University of Oslo (reported work in anthracis)
- Department of Infectious Diseases, Ullevål University Hospital, Oslo

Poland:

- Institute of Infectious and Parasitic Diseases, Medical University of Warsaw, ul. Wolska 37, Warsaw
- Center of Microbiology and Infectious Diseases, National Institute of Public Health, Chelmska Street 30/34, 00-725 Warsaw iletowska@cls.edu.pl
- Clinic of Parasitic and Tropical Diseases, Institute of Microbiology and Infectious Diseases, University of Medical Sciences, 60-355 Poznań
- Department of Infectious Diseases, Institute of Internal Diseases, Medical University of Gdansk, 80-214 Gdansk
- Department of Microbiology, Institute of Infectious and Invasive Diseases, Agricultural Academy, Lublin
- Department of Bacteriology, National Institute of Hygiene, Chocimska Street 24, 00-791 Warsaw rgierczynski@pzh.gov.pl (reported work in anthracis)

Romania:

- Molecular Diagnostics Laboratory, Prof. Dr. Matei Bals' Institute for Infectious Diseases, Str. Calistrat Grozovici, nr.1, sector 2, 021105 Bucharest

Russia:

- **State Research Center of Virology and Biotechnology (Vektor), BSL-4**, Koltsovo, Novosibirsk. Other BSL-4 reported to have been dismantled
- State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, jo.meis@gmail.com (reported work in hemorrhagic fevers)
- Research Institute for Plague Control, Rostov-on-Don. (reported work in hemorrhagic fevers)
- D. I. Ivanovsky Institute of Virology RAMS, Moscow dk_lvov@mail.ru (reported work in hemorrhagic fevers)
- State Medical Academy, Kirov. (reported work in hemorrhagic fevers)
- Daghestan Station for Plague Control, Makhachkala (reported work in hemorrhagic fevers)
- Territorial Sanitary and Epidemiological Surveillance Center, Research Institute for Plague Control, Stavropol (reported work in hemorrhagic fevers and anthracis)
- Department of Pathology, Tuberculosis and Pulmonary Diseases Unit, Hospital 40, Ekaterinburg (reported work in anthracis)

Singapore:

- **Defence Science Organization (DSO) National Laboratories, BSL-4**, Singapore. **Also a mobile BSL-4 facility**
- Department of Infectious Diseases, Communicable Disease Centre, Tock Seng Hospital jenny.low@sgh.com.sg
- Virology Group Defence Medical and Environmental Research Institute, DSO National Laboratories (reported work in Dengue fever)
- Genome Institute of Singapore, Biopolis (reported work in Dengue fever)
- Programme in Infectious Diseases, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore
- Environmental Health Institute, National Environment Agency Christina_LIEW@nea.gov.sg (reported work in Dengue virus)
- Novartis Institute for Tropical Diseases (NITD), Chromos
- Vector Control and Research Department, Ministry of the Environment chung_youne_kow@env.gov.sg (reported work in Dengue virus)

South Africa:

- **National Institute for Communicable Diseases (NICD), National Institute for Virology (NIV), Special Pathogens Unit (SPU)**, BSL-4, Grahamstown http://www.scienceinafrica.co.za/2002/september/nicd.htm
- Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Anzio Road, Observatory Cape Town GJKOTW01@gmail.com
- Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, Cape Town tihana.bicanic@stgeorges.nhs.uk
- National Institute for Virology, Sandringham (reported work in viral hemorrhagic fevers)
- Special Pathogens Unit, National Institute for Communicable Diseases, Sandringham felicityb@nicd.ac.za (reported work in hemorrhagic fevers)
- South African Institute for Medical Research, Johannesburg KEITHK@mail.saimr.wits.ac.za (reported work in emerging infectious diseases)

South Korea:

- Division of Infectious Diseases, Korea University, College of Medicine, Seongbuk-gu, Seoul
- World Health Organization Collaborating Centre for Virus Reference and Research, Institute for Viral Diseases, Korea University, Seoul

- Department of Internal Medicine, College of Medicine, Seoul National University (reported work in hemorrhagic fever)
- Bio-Safety Research Institute and College of Veterinary Medicine, Chonbuk National University, Jeonju

Spain:

- Research Unit on Infectious Diseases and Mycology (URMIM), Municipal Institute for Medical Research (IMIM), Autonomous University of Barcelona
- Service of Infectious Diseases, Hospital Carlos III, Carlos III Institute of Health, Madrid
- Clinic Institute of Infectious Diseases and Immunology, IDIBAPS, Hospital Clínic, Faculty of Medicine, University of Barcelona fgarcia@medicina.ub.es
- Department of Clinical Microbiology and Infectious Diseases, Hospital Ramón y Cajal, Madrid
- Department of Infectious Diseases, Hospital de Bellvitge, Universidad de Barcelona
- Unidad de Medicina Tropical, Servicio de Enfermedades Infecciosas, Hospital Ramón y Cajal, Madrid rlopezvelez.hrc@salud.madrid.org

Sweden:

- **Swedish Institute for Infectious Disease Control, Karolinska Institutet, BSL-4**, Solna
- Department of Infectious Diseases, Kalmar County Hospital, SE-391 85 Kalmar mats.haglund@ltkalmer.se
- Department of Medicine, Division of Infectious Diseases, Center for Infectious Medicine, Karolinska Institutet at Karolinska University Hospital, Huddinge jonas.sunden-cullberg@ki.se

Switzerland:

- **Institute of Virology and Immunoprophylaxis** (IVI), **BSL-4**, Mittelhäusern
- Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, 3010 Bern
- Institute of Parasitology, Division of Infectious Diseases University Hospital, University of Zürich, Winterthurerstrasse 266a, CH-8057 Zürich
- Divisions of Infectious Diseases, Central Institute of the Valais Hospitals vera.vongunten@ichv.vsnet.ch
- Travel and Migration Medicine Unit, Department of Community Medicine, Geneva University Hospital (reported work in Dengue fever)
- Infection Control Programme and Medical Intensive Care Unit, Department of Internal Medicine, University of Geneva Hospitals, Geneva (reported work in Ebola)

- UEPP, IUMSP, Université de Lausanne. huynhdophi@iname.com (reported work in emerging infectious diseases)

Taiwan:

- **Preventive Medical Institute, Ministry of National Defense, BSL-4**, Taiwan
- **Kwen-yang Laboratory, Center of Disease Control, Department of Health ROC, BSL-4**, Taiwan
- Institute of Microbiology and Biochemistry, National Taiwan University, Taipei

Thailand:

- Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi
- National Center for Genetic Engineering and Biotechnology and Emerging Infectious Disease Program (EIDP)

Turkey:

- Gulhane Military Medical Academy, Ankara
- NBC School, Turkish Armed Forces, Kucukyali (Istanbul)
- Etlik Veterinary Control and Research Institute, Ankara
- Department of Infectious Diseases, Ankara Numune Education and Research Hospital, Ankara onderergonul@yahoo.com

United Kingdom:

- **Porton Down, DSTL**, BSL-4, United Kingdom
- **Health Protection Agency, Centre for Infections, Viral Zoonosis unit**, BSL-4, Colinade, United Kingdom
- **National Institute for Medical Research,** BSL-4, London, United Kingdom. Under construction
- Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Cambridgc
- Division of Virology, National Institute for Biological Standards and Control, Blanchc Lanc, South Mimms, Potters Bar, Hertfordshire jrobertson@nibsc.ac.uk
- Institute of Molecular Medicine, Epidemiology and Cancer Research, University of Leeds, St James's University Hospital, Beckett Street, Leeds a.w.morgan@leeds.ac.uk

- Centre for Infectious Disease, Institute of Cell and Molecular Science, Queen Mary, University of London, 4th Floor, 51-53 Bart's Close, St Bart's Hospital, West Smithfield, London j.l.chapman@qmul.ac.uk
- Infectious Diseases and Microbiology Unit, Institute of Child Health, 30 Guilford Street, London
- The University of Nottingham, Institute of Infection, Immunity and Inflammation, School of Molecular Medical Sciences, Division of Microbiology and Infectious Diseases, Queens Medical Centre, West Block, Nottingham richard.brown@nottingham.ac.uk
- Centre for Infectious Diseases, Institute for Cell and Molecular Science, 4 Newark Street, Whitechapel, London m_quinlivan@hotmail.com
- MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, Queen Square, London
- Wohl Virion Centre, Windeyer Institute of Medical Sciences, University College London, 46 Cleveland Street, London
- The Edward Jenner Institute for Vaccine Research, Compton, Berkshire elma.tchilian@jenner.ac.uk
- Centre for Infectious Diseases and International Health, Windeyer Institute of Medical Sciences, University College London, London spigelman@btinternet.com

United States of America:

- Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), **BSL-4**, Hamilton, Montana http://www3.niaid.nih.gov/about/organization/dir/rml/
- Southwest Foundation for Biomedical Research, **BSL-4**, San Antonio, Texashttp://www.sfbr.org/pages/virology_projects.php?p=16 (accessed January 2008)
- **National Bio and Agro-Defense Facility (NBAF), Department of Homeland Security**, BSL-4, location to be determined; construction planned for 2010
- **National Biodefense Analysis and Countermeasures Center (NBACC), Department of Homeland Security**, BSL-4, Fort Detrick, Maryland. Under construction
- **National Emerging Infectious Diseases Laboratory (NEILD)**, BSL-4, Boston, Massachusetts Under construction
- **United States Army Medical Research Institute of Infectious Diseases (USAMRIID)**, BSL-4, Fort Detrick, Frederick, Maryland http://www.usamriid.army.mil/ (accessed July 2007)
- **National Institutes of Health BSL-4 Lab, Building 41A,** BSL-4, Bethesda, Maryland Reportedly operates as a BSL-3 facility and a BSL-4 training facility

- **National Institutes of Health BSL-4 Lab, Twinbrook III Building,** BSL-4, Rockville, Maryland
- **Integrated Research Facility, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH),** BSL-4, Fort Detrick, Frederick, Maryland Under construction
- **Division of Consolidated Laboratory Services**, BSL-4, Department of General Services of the Commonwealth of Virginia, Richmond, Virginia http://dcls.dgs.state.va.us/Services/Immunology.aspx The laboratory is now being challenged to develop rapid methods for detecting recent threats to public health such as West Nile Virus, SARS, Monkeypox, and with the threat of terrorism, Smallpox.
- **Centers for Disease Control (CDC)**, BSL-4, Atlanta, Georgia
- **Centre for Biodefense and Emerging Infectious Diseases**, BSL-4, University of Texas Medical Branch Galveston, Texas http://www.utmb.edu/CBEID/BSL4.htm (accessed January 2008). This BSL-4 is devoted to the study of tropical and emerging infections and also will serve as a key component in the nation's fight against bioterrorism. The site contains a link for a virtual tour of the lab.
- **Centre for Biotechnology and Drug Design,** BSL-4, Georgia State University, Atlanta, Georgia http://www.cas.gsu.edu/units/default.aspx?unit=biotech&section=viral (accessed September 2007). The facility is available to local skilled and experienced scientists to work within the biocontainment condition.